

Temperature Effects on Threshold Counterion Concentration to Induce Aggregation of fd Virus

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We seek to elucidate the dominant mechanism of attractive interaction between like-charged biopolymers by measuring the temperature dependence of the critical divalent counterion concentration (C_c) for the aggregation of fd viruses. A decrease in either temperature or the dielectric constant alone causes a decrease in C_c , providing evidence for the Wigner crystal model. Surprisingly, the effects of these two parameters can be combined so that C_c is expressed as a function of a single parameter: the Bjerrum length. C_c decreases exponentially as the Bjerrum length increases, suggesting that an energetic balance between the entropic effect of counterions and the counterion mediated attractive interaction gives rise to the onset of bundle formation.

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Experiments on a variety of charged biopolymers, such as DNA [1,2], *F*-actin [3–5], microtubules, and bacteriophages [6,7] show that they can self-assemble to form bundles in the presence of counterions. The attractive interaction that holds the like-charged polymers together, which is often referred to as like-charge attraction, requires a treatment beyond the prediction of mean-field theory. During the past few decades, there have been intensive theoretical investigations to explore the mechanism of counterion induced attractive interaction between polyelectrolytes [8–16]. Although other types of interactions such as hydration [8,16] and depletion [17] are contributing factors, it has been established that the electrostatic interaction between the polyelectrolytes and their correlated counterions [4,9–15] is the major cause of this like-charge attraction.

Two possible mechanisms of counterion correlation have been proposed to induce attractive interaction between rodlike polyelectrolytes. Thermal fluctuations create transient regions of high and low counterion densities along the polyelectrolytes, which are typically simplified to be charged lines each with a thin layer of condensed counterions [18]. When two parallel polyelectrolytes approach each other, an attractive force is induced by their transient complementary counterion density profiles. This mechanism is referred to as the Oosawa model. Alternatively, the counterions may correlate with each other in their positions on the surface of the polyelectrolytes. A representative picture of the attractive interaction induced by positional correlations of counterions is provided by the Wigner crystal model [11,19]. In this model, the condensed counterions form Wigner crystals on the polyelectrolyte's surface at the low temperature limit. Cross correlation of counterions occurs when the distance between two polyelectrolytes decreases to the lattice constant of the Wigner crystals. The cohesive energy of the Wigner crystals results in an attractive interaction and aggregation of the like-charged rods [11,19]. Although the Wigner crystal model is a zero temperature approximation, additional analytical treatment and computer simulations have extended the

concept to finite temperatures by treating the counterions as strongly correlated liquid [10,20]. For simplicity, we refer to this type of theoretical treatment as the Wigner crystal model.

While both models predict an attractive electrostatic interaction, their difference in correlation mechanism gives rise to opposite effects of temperature (T) on the strength of the attraction. The Oosawa model originates in the process of thermal agitation. A higher T leads to larger fluctuations in the counterion density. A stronger correlation is therefore predicted under a higher T [9,10,18]. Conversely, the Wigner crystal model predicts a weaker correlation under a higher T , since the thermal motion of counterions destroys the lattice structure of the ionic crystals [10,11,15,21].

Although many experiments show that like-charge attraction originates in electrostatic interactions, most published works have not been able to distinguish between different models. Raspaud *et al.* show the electrostatic nature of the attractive interaction between DNA [2]. Their results were interpreted by the ion-bridging model, which is different from the Oosawa model, but similar to the Wigner crystal model [12]. Using small angle x-ray scattering, Angelini *et al.* observed a one-dimensional counterion density wave (CDW) in the bundles of *F*-actin [4]. Instead of providing a proof for either the Oosawa model or the Wigner crystal model, their results provide a transitional picture between them.

In this Letter, we present the effects of T on the critical concentration of counterions C_c , using MgCl_2 and CaCl_2 to induce bundle formation of bacteriophage fd in ethanol and water mixtures. Ethanol was added to the solution mainly for the purpose of changing the dielectric constant (ϵ). The measured effect of T on C_c , which was isolated from the effect of ϵ , provides experimental evidence for the Wigner crystal model. The Bjerrum length (l_B) is found, for the first time, to be the sole parameter that controls C_c . A plausible thermodynamic model is proposed for the aggregation of fd, which is consistent with the finding that C_c drops exponentially as a function of l_B .

Bacteriophage fd is a rodlike polyelectrolyte approximately 880 nm in length and 6.6 nm in diameter. There are approximately 2700 copies of coat proteins arranged on the virus surface. At neutral pH , each coat protein contributes four net negative charges on the surface of a virus. A virus therefore has a linear charge density of approximately 12.5 e/nm, quite close to that of a double stranded DNA. Because of this high charge density, the lateral aggregation of fd viruses can be induced by divalent counterions such as Mg^{2+} and Ca^{2+} [6,7]. Aggregation of fd viruses was detected by measuring the scattered light intensity at a fixed angle of 90° with a PERKIN ELMER LS-5 luminescence spectrometer. 800 μ l of 0.1 mg/ml [22] fd virus was added to a rectangular cuvette of 10 mm path length and 5 mm width. The scattering intensity was recorded when the reading became stable following the addition of a stock solution of concentrated $CaCl_2$ or $MgCl_2$. The sample temperature was controlled using a water bath (ISOTEMP 1006S, Fisher Sci., Inc.) connected to the sample holder. When increasing the counterion concentration, an abrupt increase in the light scattering intensity was observed at the critical concentration C_c . The C_c was defined as the divalent concentration where at least a tenfold increase in the total scattering intensity was observed.

The threshold concentration was measured in the absence of ethanol at $T = 10, 20, 30,$ and $40^\circ C$. The data in Fig. 1(a) shows that the threshold concentrations for both $MgCl_2$ and $CaCl_2$ decrease as T increases. The ϵ for the solution, which is approximately equal to that of water, also varies as a function of T , $\epsilon(T) = a + bT + cT^2 + dT^3$, where a, b, c, d are empirical constants [23]. The calculated ϵ is shown to decrease from 83.9 to 73.2 when T increases from 10 to $40^\circ C$.

Within our experimental range ($T \leq 50^\circ C$), there is no structural change of the viruses. The fd viruses survive at T up to $90^\circ C$ [24,25], suggestive of their robust structural integrity. The changes in ϵ with increasing T , however, do affect our measurements, since a change in ϵ directly affects the strength of electrostatic interaction.

To explore the effect of ϵ on the aggregation of fd virus, the C_c was measured for samples in ethanol and water mixtures under a fixed temperature $T = 20^\circ C$. The data in Fig. 1(b) show that as the amount of ethanol in solution increases up to 18.5 v/v%, C_c decreases monotonically.

Adding ethanol effectively decreases the ϵ of the solvent. For a mixture of ethanol and water, ϵ depends on the ethanol concentration as $\epsilon(T) = (1 - c_e)\epsilon_w(T) + c_e\epsilon_e(T)$ [26], where $\epsilon_w(T)$ and $\epsilon_e(T)$ are dielectric constants of water and ethanol at T , respectively, and c_e is the volume fraction of ethanol in the solution. At $20^\circ C$, $\epsilon_w = 80.37$ and $\epsilon_e = 25.00$, the calculated ϵ of the bulk solution is shown in Fig. 1(b) to decrease with c_e .

The nonpolar ethanol molecules are known to be excluded from the high electric field region, since its polarizability is much smaller than that of water. One can then argue that the local ϵ near the charged virus surface might

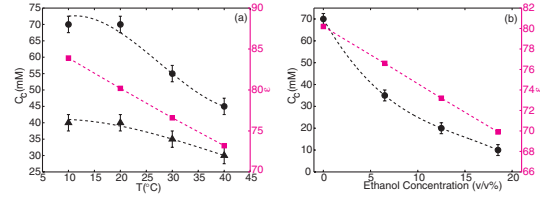


FIG. 1 (color online). An increase in temperature (T) leads to a decrease in dielectric constant (ϵ) of the solvent, thus causing C_c to decrease. (a) When the ϵ of the solvent is not a fixed value, the C_c for either $MgCl_2$ (●) or $CaCl_2$ (▲) decreases with increasing T . As T increases, ϵ (■) drops. (b) C_c for $MgCl_2$ (●) decreases with the amount of ethanol at $20^\circ C$. Addition of ethanol leads to a decrease in ϵ (■).

be different from that in the bulk ethanol/water mixture. Arscott *et al.* have shown by the use of various alcohols with dielectric constants smaller than that of water, however, that the threshold hexammine cobalt (III) concentration to induce DNA condensation is primarily a function of ϵ of the solvent [1]. In the presence of aminocarboxylic acid, which is known to increase ϵ of the solvent, the C_c of multivalent counterions needed to induce DNA condensation has also been shown to increase with increasing ϵ [27]. Moreover, results of recent studies show that ϵ of the solvent is a key factor in determining the conformational behavior of single DNA molecules [28]. These experiments indicate that, although there is debate on whether the added ethanol changes the local ϵ close to the virus surface, we can practically interpret the decrease of C_c as a result of increased electrostatic interaction due to the decrease in ϵ .

To define the net effect of T on C_c , the ϵ was fixed under different temperatures by adding appropriate amounts of ethanol. Both curves in Fig. 2(a) show that an increase in T causes C_c to increase under fixed ϵ . At $\epsilon = 73.2$, C_c for $MgCl_2$ was measured to increase from 10 to 45 mM as the temperature rose from 10 to $40^\circ C$. At $\epsilon = 69.9$, the C_c was observed to increase monotonically from 5 to 40 mM as T increased from 10 to $50^\circ C$. The curve for $\epsilon = 69.9$ is lower than the curve for $\epsilon = 73.2$. This is expected, since we know that a lower ϵ leads to a lower C_c .

Based on the observation that a decrease in either T or ϵ alone leads to a lower C_c , the drop of C_c in Fig. 1(a) can be interpreted as the net effect of an increase in T and a decrease in ϵ , which is induced by the rising T . Furthermore, the l_B , which is defined as $l_B = e^2/4\pi\epsilon\epsilon_0k_B T$, appears to be the single parameter that combines the effects of T and ϵ to control the C_c . By fixing the Bjerrum length, C_c was measured as a function of T . As shown in Fig. 2(b), the measured values for C_c were 45 and 40 mM for $l_B = 7.28$ and 7.38, respectively. No observable effects of T on C_c was detected at a constant Bjerrum length. The change in l_B has been observed to drastically affect C_c . By plotting C_c as a function of l_B , Fig. 3 shows that C_c decreases exponentially. The logarithm of C_c (omitting the unit in mM) fits to a linear function of l_B : $\ln C_c = -Al_B + B$, with

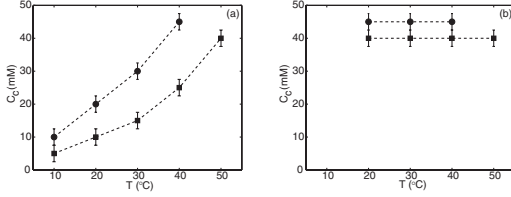


FIG. 2. Effect of temperature (T) on C_c under fixed dielectric constants (ϵ) and fixed l_B . (a) At $\epsilon = 73.2$ (●) and $\epsilon = 69.9$ (■), C_c increases with increasing T . (b) When the solution ϵ is adjusted to fix l_B , C_c does not vary with T . At fixed Bjerrum lengths, C_c is 45 mM with $l_B = 7.28$ (●), and 40 mM with $l_B = 7.38$ (■).

$A = 1.89 \text{ \AA}^{-1}$, $B = 17.6$ for MgCl_2 and $A = 2.09 \text{ \AA}^{-1}$, $B = 18.5$ for CaCl_2 , respectively.

The Bjerrum length is often used to quantify the strength of counterion correlations through a coupling parameter $\Gamma = 2\pi z^3 l_B^3 \sigma$, where z is the valence of counterions and σ represents the surface charge density of polyelectrolytes [20]. A larger l_B indicates a stronger counterion correlation. Therefore, our result of a lower C_c observed at a larger l_B is consistent with the picture that a stronger correlation leads to a lower threshold concentration.

By adding charge density fluctuations to the mean-field theory, Oosawa derived an attractive force between charged lines [10,18]. The predicted attractive interaction is stronger at a higher T , which is contrary to our experimental findings. This suggests that the thermal fluctuation of counterion density is not the dominant mechanism for like-charge attraction.

The Wigner crystal model takes into account the strong correlation between counterions. Prior to aggregation, each fd virus is surrounded by a layer of condensed counterions, which partially compensates the charge of the fd virus. As a result of the decrease in fd surface charge, the viruses are able to move close to each other by means of Brownian motion, since the Coulomb repulsion is reduced. When the distance between two viruses is small enough, the counterions around each virus start to correlate with each other. This correlation creates an attractive interaction between

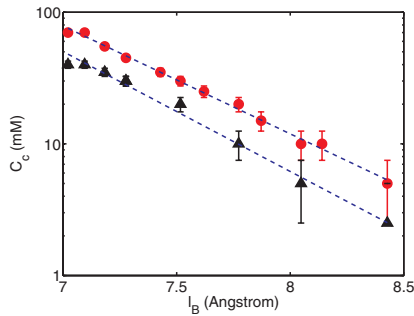


FIG. 3 (color online). C_c as a function of l_B . Results for MgCl_2 (▲) and CaCl_2 (●) indicate that C_c decreases exponentially with increasing l_B . Dashed lines are fits to the experimental data with $\ln C_c = -A l_B + B$.

the two viruses. Each counterion gains an energy of G_{corr} from this attractive interaction. Since $\Gamma \approx 14.8$ for fd virus at room temperature and $\epsilon = 80$, the condensed counterions can be treated as a strongly correlated liquid. The long range order of counterions, which should be observable using low angle x-ray scattering, does not occur in the strongly correlated liquid. Nonetheless, the counterion correlation in the strongly correlated liquid keeps the characteristics of the Wigner crystal model. G_{corr} is therefore estimated as the energy of interaction between a counterion and its Wigner-Seitz cell [11],

$$G_{\text{corr}} = \frac{z}{2b} (zb/r)^{3/4} l_B k_B T, \quad (1)$$

where b is the charge spacing on the polyelectrolyte when considering it as a charged line, and r is the radius of a virus. Within a bundle of fd viruses, G_{corr} is estimated to be $0.13 l_B k_B T$ per counterion using $b = 0.8 \text{ \AA}$ and $r = 33 \text{ \AA}$.

The strong correlation also drives more counterions from the bulk solution into the space between two viruses [12,13]. In addition to increasing the attractive free energy, these extra counterions roughly neutralize the residual charge of the fd viruses, thus diminishing the Coulomb repulsion. The entropy loss of these counterions, however, incurs an energy penalty. Assuming that a fraction γ of all counterions in the condensation layer are the extra ones, the average energy penalty per counterion is

$$G_R = \gamma k_B T \ln(C_0/C), \quad (2)$$

where C_0 is the counterion concentration in the condensation layer.

Aggregation of fd viruses occurs when the energy gain is larger than the energy penalty, i.e., $G_{\text{corr}} - G_R > 0$. Using Eqs. (1) and (2), the critical concentration C_c can be calculated as

$$\ln C_c = -\frac{z^{7/4} b^{1/4}}{2\gamma r^{3/4}} l_B + \ln C_0. \quad (3)$$

From Eq. (3), γ can be estimated by setting the fitting parameter as $A = \frac{z^{7/4} b^{1/4}}{2\gamma r^{3/4}}$. Based on the values of $A = 1.89 \text{ \AA}^{-1}$ and 2.09 \AA^{-1} , we obtain $\gamma \approx 6\%$ and 7% for CaCl_2 and MgCl_2 , respectively. When calculated from the Manning counterion condensation theory, approximately 94% of the surface charge of a virus is neutralized by divalent counterions at the onset of aggregation. These predictions suggest that the viruses are essentially totally neutralized when they aggregate.

The second term in Eq. (3) contributes to the fitting parameter B . The counterion concentration in the condensation layer has been measured to be on the order of 1 M [29]. Using $C_0 = 3000 \text{ mM}$ for both Mg^{2+} and Ca^{2+} , the parameter B is estimated to be 8.01. This value is much smaller than the fitting result. The discrepancy likely originates from the change in the counterion hydration state. A counterion loses its waters of hydration when it resides in the condensation layer. The ^{25}Mg NMR measurements of

F-actin solutions indicate that only a small fraction of the condensed Mg^{2+} lose their waters of hydration [30]. If a small portion, say 10%, of the extra counterions lose their waters of hydration, an energy penalty of approximately $10k_B T$ is expected for each extra counterion, since the energy penalty for a Mg^{2+} or Ca^{2+} ion to lose all of its waters of hydration is on the order of $100k_B T$ [31]. When taking into account the hydration effect, G_R is rewritten as, $G_R = \gamma k_B T (\ln C_0/C + 10)$. Accordingly, the constant term in the fitting shown in Fig. 3 is translated to $B = \ln C_0 + 10 \approx 18$, which is close to the values from the fitting. This estimate suggests that partial loss of hydration contributes to the change in G_R , thereby affecting the C_c of multivalent counterions for bundle formation. Additionally, G_R may also be affected by a change in counterion activity, which varies with the ion concentration and the added alcohol. The effect of such a change on G_R is expected to be small as the activity enters into the argument of the logarithm term in Eq. (2). The effect of counterion activity, however, is not analyzed due to the lack of the data on activities of Mg^{2+} and Ca^{2+} in alcohol and water mixtures.

In the DNA condensation experiments [8], the threshold concentration of Mn^{2+} ions to induce DNA condensation has also been reported to decrease with increasing T . This phenomenon is similar to the result in Fig. 1(a). When taking into account the variation in solution ϵ , the data in Ref. [8] indicate an exponential decay of C_c with increasing l_B as well. Instead of discussing the effect of variations in dielectric constant, the data was interpreted by assuming that the attractive force originates in the release of excess water from the polyelectrolyte surfaces [8,16]. It is interesting to note that the analysis based on the release of water molecules also predicts an extra binding of counterions in the process of DNA condensation [16]. The coexistence of distinct physical models reminds us of the nondefinitiveness of the current state of understanding of the subject.

In summary, we have studied the thermodynamic effect of like-charge attraction by measuring the effects of T on threshold concentrations for divalent counterions to induce bundle formation of fd viruses. The decrease in C_c as a function of the added ethanol has been attributed to the effect of the lowered ϵ . Assuming that the major effect of ethanol in our experiments was tuning ϵ , we found that C_c varies with the Bjerrum length as a single variable. Our results show for the first time that there is a linear relationship between $\ln C_c$ and l_B , which is well interpreted using the Wigner crystal model, supporting the assertion that it is applicable at finite temperatures with the notion of a strongly correlated liquid.

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- [1] P. G. Arscott, C. Ma, J. R. Wenner, and V. A. Bloomfield, *Biopolymers* **36**, 345 (1995).
- [2] E. Raspaud, M. O. de la Cruz, J. L. Sikorav, and F. Livolant, *Biophys. J.* **74**, 381 (1998).
- [3] J. X. Tang and P. A. Janmey, *J. Biol. Chem.* **271**, 8556 (1996).
- [4] T. E. Angelini, H. Liang, W. Wriggers, and G. C. L. Wong, *Proc. Natl. Acad. Sci. U.S.A.* **100**, 8634 (2003).
- [5] J. C. Butler, T. Angelini, J. X. Tang, and G. C. L. Wong, *Phys. Rev. Lett.* **91**, 028301 (2003).
- [6] A. P. Lyubartsev, J. X. Tang, P. A. Janmey, and L. Nordenskiöld, *Phys. Rev. Lett.* **81**, 5465 (1998).
- [7] J. X. Tang, P. A. Janmey, A. Lyubartsev, and L. Nordenskiöld, *Biophys. J.* **83**, 566 (2002).
- [8] D. C. Rau and V. A. Parsegian, *Biophys. J.* **61**, 260 (1992).
- [9] B.-Y. Ha and A. J. Liu, *Phys. Rev. Lett.* **79**, 1289 (1997).
- [10] N. Grønbech-Jensen, R. J. Mashl, R. F. Bruinsma, and W. M. Gelbart, *Phys. Rev. Lett.* **78**, 2477 (1997).
- [11] B. I. Shklovskii, *Phys. Rev. Lett.* **82**, 3268 (1999).
- [12] A. Y. Grosberg, T. T. Nguyen, and B. I. Shklovskii, *Rev. Mod. Phys.* **74**, 329 (2002).
- [13] T. T. Nguyen, I. Rouzina, and B. I. Shklovskii, *J. Chem. Phys.* **112**, 2562 (2000).
- [14] F. J. Solis and M. O. de la Cruz, *Phys. Rev. E* **60**, 4496 (1999).
- [15] J. J. Arenzon, J. F. Stilck, and Y. Levin, *Eur. Phys. J. B* **12**, 79 (1999).
- [16] A. Hultgren and D. C. Rau, *Biochemistry* **43**, 8272 (2004).
- [17] J. X. Tang, T. Ito, T. Tao, P. Traub, and P. A. Janmey, *Biochemistry* **36**, 12600 (1997).
- [18] F. Oosawa, *Polyelectrolytes* (Marcel Dekker, New York, 1971).
- [19] I. Rouzina and V. A. Bloomfield, *J. Phys. Chem.* **100**, 9977 (1996).
- [20] A. G. Moreira and R. R. Netz, *Phys. Rev. Lett.* **87**, 078301 (2001).
- [21] B. I. Shklovskii, *Phys. Rev. E* **60**, 5802 (1999).
- [22] The dilute limit of fd virus concentration is below 0.08 mg/ml. We performed measurements with fd concentration in the range of 0.01–1 mg/ml and found the C_c to be constant.
- [23] D. R. Lide, *CRC Handbook of Chemistry and Physics* (CRC Press, Boca Raton, FL, 2002), 82nd ed.
- [24] R. W. Williams, A. K. Dunker, and W. L. Peticolas, *Biochim. Biophys. Acta* **791**, 131 (1984).
- [25] X. Ji, J. Oh, A. K. Dunker, and K. W. Hipps, *Ultra-microscopy* **72**, 165 (1998).
- [26] P. S. Neelakantaswamy, B. V. R. Chowdari, and A. Rajaratnam, *J. Phys. D: Appl. Phys.* **16**, 1785 (1983).
- [27] E. J. Cohn and J. T. Edsall, *Proteins, Amino Acids and Peptides as Ions and Dipolar Ions* (Reinhold, New York, 1943), p. 145.
- [28] D. Baigl and K. Yoshikawa, *Biophys. J.* **88**, 3486 (2005).
- [29] G. S. Manning, *Q. Rev. Biophys.* **II 2**, 180 (1978).
- [30] W. Xian, J. X. Tang, P. A. Janmey, and W. H. Braunlin, *Biochemistry* **38**, 7219 (1999).
- [31] M. Pavlov, P. E. M. Siegbahn, and M. Sandström, *J. Phys. Chem. A* **102**, 219 (1998).