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

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Abstract

We seek to determine the mechanism of like-charge attraction by measuring the temperature dependence of critical divalent counterion concentration ($C_c$) for the aggregation of fd viruses. We find that an increase in temperature causes $C_c$ to decrease, primarily due to a decrease in the dielectric constant ($\varepsilon$) of the solvent. At a constant $\varepsilon$, $C_c$ is found to increase as the temperature increases. The effects of $T$ and $\varepsilon$ on $C_c$ can be combined to that of one parameter: Bjerrum length ($l_B$). $C_c$ decreases exponentially as $l_B$ increases, suggesting that entropic effect of counterions plays an important role at the onset of bundle formation.

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Multivalent counterion induced aggregation of polyelectrolytes has been observed in a variety of systems such as DNA, F-actin and bacteriophages [1, 2, 3, 4]. During the past decades, there have been intensive theoretical investigations to elucidate the mechanism of counterion induced attractive interaction between polyelectrolytes [5, 6, 7, 8, 9, 10, 11, 12, 13]. Although other types of interactions such as hydration [12, 13] and depletion [14] are contributing factors, it has been established that the electrostatic between the polyelectrolytes and their correlated counterions [5, 6, 7, 8, 9, 10, 11, 15] is the major cause of like-charge attraction.

There are, however, two possible ways, in which the condensed counterions on different polyelectrolytes correlate with each other. Thermal fluctuations create transient regions of high or low counterion density along the polyelectrolytes, which are typically simplified to be charged lines each with a thin layer of condensed counterions [16]. When two parallel polyelectrolytes get close to each other, an attractive force is induced by their transient complementary counterion density profiles. The attractive force induced by the long wavelength counterion fluctuations has been predicted to be proportional to temperature using the mean field theory [16]. Alternatively, the counterions may correlate with each other in their positions on the surfaces of polyelectrolytes. A representative picture of the attractive interaction induced by positional correlations of counterions is provided by the Wigner crystal model [7]. In this model, condensed counterions form Wigner crystals on the polyelectrolyte’s surface at $T = 0$ K. Cross correlation of counterions occurs when the distance between two polyelectrolytes decreases to the lattice constant of the Wigner crystals. It is the cohesive energy of the Wigner crystals that results in attractive interaction and aggregation of like-charged rods [7, 17]. Since thermal fluctuation diminishes the structural order of counterions, this model predicts a stronger attractive interaction at a lower temperature [16].

Recent computer simulation results suggest that the Wigner crystal model captures the physics of like-charge attraction better than the thermal fluctuation model [6], since it takes into account the strong correlations between individual counterions. However, the Wigner crystal model is a zero temperature approximation. It is uncertain whether the model works under high temperatures, even though it has been suggested in the theoretical work that the short range order of counterions survives at high temperature [6, 8, 18]. Since the two theoretical models predict opposite temperature effects on attractive interactions, the aggregation of polyelectrolytes under different temperatures is expected to directly reveal
the dominant mechanism of like-charge attraction.

In this paper, we present the results of temperature effects on critical concentration of counterions $C_c$, using MgCl$_2$ and CaCl$_2$ to induce bundle formation of bacteriophage fd. We found that $C_c$ increases with increasing temperature under a certain dielectric constant. The effect of the dielectric constant on $C_c$ has been measured as well. Under a fixed temperature, $C_c$ decreases with decreasing $\varepsilon$. Noting the similar effects of $T$ and $\varepsilon$ on threshold concentration, we speculate that $C_c$ is actually determined by the Bjerrum length ($l_B$). The result that $C_c$ doesn’t change with temperature at a constant $l_B$ validates our speculation. The thermodynamics for aggregation of fd virus is investigated using the result that $C_c$ drops exponentially as a function of $l_B$. The effect of hydration of counterions can be assessed from the prefactor in the exponential expression. Finally, based on the result that stronger counterion correlations lead to lower threshold concentration, we conclude that our results support the Wigner Crystal model.

Bacteriophage fd is a rod-like polyelectrolyte of approximately 880 nm in length, and 6.6 nm in diameter. There are approximately 2,700 copies of coat protein arranged crystallographically on the virus surface. At neutral pH, each coat protein contributes four net negative charges on the surface of a virus. Thus, a virus has a linear charge density of approximately 12.5 e/nm. When a virus is simplified to a linear array of charges, the charge spacing would be $b = 0.8$ Å. The fd viruses are highly charged so that their lateral aggregation can be induced by divalent counterions such as Mg$^{2+}$ and Ca$^{2+}$ [18, 19]. The viruses survive at temperatures up to 90°C [20, 21]. Therefore, within our experimental range ($T \leq 50$ °C), the results are not expected to be due to the changes in the physical parameters of virus.

Aggregation of fd virus is detected by measuring the scattering light intensity at a fixed angle of 90°, with a PERKIN ELMER LS-5 luminescence spectrometer. 800 µl 0.1 mg/ml bacteriophage fd was added to a rectangular cuvette of 10 mm path length and 5 mm width. Scattering intensity was measured when the solution reached its steady state following the addition of a stock solution of concentrated CaCl$_2$ or MgCl$_2$. Sample temperature was controlled using a ISO TEMP 1006S water bath (Fisher Sci Inc.) connected to the sample holder. When increasing the counterion concentration, an abrupt increase in the light scattering intensity was noted at a critical concentration $C_c$. The $C_c$ was defined at the divalent concentration where at least a 20-fold increase in the total scattering intensity was observed.
To find out the temperature effect on threshold concentration, we first measured the threshold concentration under different temperatures without other manipulation of solution properties. Next, the effect of solution dielectric constant on $C_c$ was measured under a constant temperature of 20 °C. Finally, $C_c$ was measured at different temperatures with the dielectric constant fixed.

Fig.1(a) shows threshold concentrations of MgCl$_2$ and CaCl$_2$ as functions of the sample temperature. $C_c$ for both MgCl$_2$ and CaCl$_2$ decreases with increasing temperature. However, the temperature changes of a solution also results in changes of its dielectric constant. The dielectric constant of the solution, which is approximately equal to that of water, under different temperatures are calculated using $\varepsilon(T) = a + bT + cT^2 + dT^3$, where $a$, $b$, $c$, $d$ are empirical constants [22]. It is shown in Fig.1(a) that the calculated $\varepsilon$ of water decreases from 83.9 to 73.2 when temperature increases from 10 to 40 °C.

Changing the dielectric constant dramatically affects $C_c$. In Fig.1(b), $C_c$ is measured under different ethanol concentrations at $T = 20$ °C. Adding ethanol to a solution effectively changes its dielectric constant. $\varepsilon$ for a mixture of ethanol and water is determined by $\varepsilon(T) = (1 - c_e)\varepsilon_w(T) + c_e\varepsilon_e(T)$ [23], where $\varepsilon_w(T)$ and $\varepsilon_e(T)$ are dielectric constants of water and ethanol at temperature $T$ respectively, and $c_e$ is the volume fraction of ethanol in the solution. At 20 °C, $\varepsilon_w = 80.37$ and $\varepsilon_e = 25.00$. According to the relation between $\varepsilon$ and $c_e$, the dielectric constant of a solution drops when its ethanol concentration increases (Fig.1(b)). The data in Fig.1(b) show that a decrease in $\varepsilon$ leads to decrease of $C_c$. Similar effect of ethanol on critical trivalent counterion concentration has been observed in DNA condensation experiments [4]. The relationship between $C_c$ and $\varepsilon$ indicates that the like-charge attraction originates from electrostatic correlations between counterions. Since the strength of counterion correlations is proportional to $1/\varepsilon$, a smaller $\varepsilon$ leads to a stronger counterion correlation, indicated by a lower $C_c$.

Since $C_c$ decreases upon the decrease of $\varepsilon$, the dependence of $C_c$ as a function of temperature under a fixed dielectric constant is required to manifest the pure temperature effect. In Fig.2, $C_c$ increases as $T$ increases under fixed dielectric constant. $\varepsilon$ of the sample is tuned to be constant under different temperatures by the addition of appropriate amounts of ethanol. At $\varepsilon = 73.2$, $C_c$ for MgCl$_2$ increases from 10 mM to 70 mM as we raise the temperature from 10 °C to 40 °C. Hence, the drop of $C_c$ in Fig.1(a) is actually the net effect of an increase in temperature and a decrease of the dielectric constant, which is induced by
the rising temperature. The curve for $\varepsilon = 69.9$ is lower than that of $\varepsilon = 73.3$. This is to be expected, because lower dielectric constants lead to lower threshold concentrations.

A decrease in either $\varepsilon$ or temperature leads to a lower threshold concentration. We speculate that the effect of temperature and dielectric constant can be combined to that of one parameter: the Bjerrum length $l_B = \frac{e^2}{4\pi \varepsilon \varepsilon_0 k_B T}$, which is often applied to quantify the importance of counterion correlations through a coupling parameter $\Xi = 2\pi z^3 l_B^3 \sigma$, where $z$ is the valence of counterions and $\sigma$ represents the surface charge density of polyelectrolytes. At strong correlation limit, i.e. $\Xi \gg 1$, the condensed counterions form two dimensional (2-D) strongly-correlated liquid or even Wigner crystals at low temperatures. $C_c$ is indeed a function of $l_B$, since it doesn’t change with temperature at a constant Bjerrum length (See Fig.2). More importantly, a lower $C_c$ is observed at a lower Bjerrum length. Thus, a stronger correlation between counterions leads to a smaller critical counterion concentration, since larger $l_B$ values indicate stronger counterion correlation.

The threshold concentration is quite sensitive to the change in Bjerrum length. In Fig.3 $C_c$ of MgCl$_2$ and CaCl$_2$ decrease exponentially as a function of $l_B$. The logarithm of $C_c$ fits to a linear function of Bjerrum length: $\ln C_c = -Al_B + B$, with $A = 1.89 \text{Å}^{-1}$, $B = 17.6$ for MgCl$_2$ and $A = 2.09 \text{Å}^{-1}$, $B = 18.5$ for CaCl$_2$, respectively. The linear relationship between $\ln C_c$ and $l_B$ indicates that the counterion entropy plays an important role in the process of aggregation. The difference in entropy for the counterions in a bundle and those in the bulk medium is approximately $\ln C_c/C_0$, where $C_0$ is the counterion concentration in the condensation layer. This entropy loss of the condensed counterions causes an increase in the energy barrier which has to be overcome by the attractive interaction induced by electrostatic correlations. Since the energy gain from electrostatic interactions is proportional to $l_B$, a linear relation between $\ln C_c$ and Bjerrum length is expected.

Based on the mean field theory approximation, the Oosawa theory predicts that the force per unit length between two polyelectrolytes as $f(R)$:

$$ f(R) = k_B T \left( \frac{1}{z^2 R l_B} - \frac{z^2 \xi^2}{(1 + z^2 \xi^2) R^2} \right) $$

where $z$ is the valence of counterions, $R$ is the distance between the long axes of two parallel polyelectrolytes, and $\xi = l_B/b$ is the Manning parameter. For highly charged polyelectrolytes, $\xi \gg 1$, $f(R)$ is attractive when the distance $R$ between two charged lines is less than $z^2 l_B$. To induce aggregation of fd virus with divalent counterions ($z = 2$) at a large
$l_B$, say $l_B = 9 \text{ Å}$, the distance between two neighboring viruses must be smaller than 4 nm. However, the distance between the long axes of two parallel viruses could not be smaller than the diameter of a virus, which is approximately 6.6 nm. Therefore, it would be impossible for divalent counterions to induce aggregations of fd virus solely based on this treatment. The Oosawa theory fails because of its mean field nature and the simplification of polyelectrolytes as charged lines.

The Wigner crystal model takes into account the strong correlation between counterions. Before 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 two viruses can move close to each other by Brownian motion, since the Coulomb repulsion is reduced. When the distance between two viruses is small enough, the counterions on different viruses start to correlate with each other. This correlation creates an attractive interaction between two viruses. Each counterion gains an energy of $G_{\text{corr}}$ from this attractive interaction. Since $\Xi \approx 14.8$ for fd virus at room temperature and $\varepsilon = 80$, the condensed counterions can be treated as a strongly correlated liquid, and $G_{\text{corr}}$ is then estimated as the energy of interaction between a counterion and its Wigner-Seitz cell [7].

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

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

The strong correlation also drives more counterions from the bulk solution into the space between two viruses [8, 9]. These extra counterions roughly neutralize the residual charge of fd viruses, thus diminishing the Coulomb repulsion. However, the entropy loss of these counterions incurs an energy penalty. Assume that a fraction $\gamma$ of all counterions in the condensation layer are the extra ones, the average energy penalty per counterion is

$$G_R = \gamma k_B T \ln C_0 / C$$

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

$$\ln C_c = -\frac{z^{7/4} b^{1/4}}{2 \gamma r^{3/4}} l_B + \ln C_0$$
From Eq. (3), $\gamma$ can be estimated by setting the fitting parameter $A = \frac{z^7/4r^{1/4}}{2\gamma^3 r^4}$. Based on the values of $A = 1.89 \text{ Å}^{-1}$ and $2.09 \text{ Å}^{-1}$, 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 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 a few molar \[C_0\] [26]. Using $C_0 = 3$ molar for both Mg$^{2+}$ and Ca$^{2+}$, the parameter $B$ is estimated to be 8.01, which is much smaller than the fitting result. The discrepancy likely originates in the change in 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 waters of hydration [27]. If a small portion, say 10%, of the extra counterions lose their waters of hydration, an energy penalty of approximately $10k_BT$ 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 $100k_BT$ [28]. When taking into account the hydration effect, \[G_R = \gamma k_BT \left( \ln C_0/C + 10 \right) \]. Thus, \[B = \ln C_0 + 10 \approx 18\], which is close to the fitting results. This estimate suggests that partial loss of hydration contributes to the change in Gibbs free energy, thereby affecting the onset concentration of multivalent counterions for bundle formation.

In the DNA condensation experiments [12], the threshold concentration of Mn$^{2+}$ ions to induce DNA condensation has also been reported to decrease with increasing temperature. The phenomenon is similar to the result in Fig. 1(a). When taking into account the variation in solution dielectric constant, the data in Ref. [12] 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 ordered water from the polyelectrolyte surfaces [12, 13]. It is interesting that the analysis based on the water exclusion also predicts an extra binding of counterions in the process of DNA condensation [13].

In summary, we have studied the thermodynamic effect of like-charge attraction by measuring the temperature effects on threshold concentrations for divalent counterions to induce bundle formation of fd viruses. Increases in both the temperature and the dielectric constant
of the solvent lead to lower critical counterion concentration. We found that $C_c$ varies as a function of Bjerrum length, which combines the effects of temperature and the dielectric constant. The Oosawa model fails to predict the aggregations of fd virus because it simplifies the polyelectrolyte rods to charged lines. The linear relation between $\ln C_c$ and $l_B$ is well interpreted using the Wigner crystal model, supporting the argument that it is applicable at finite temperatures.

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Figures
FIG. 1: Increase of temperature leads to decrease of dielectric constant ($\varepsilon$) of the solvent, thus causes $C_c$ to decrease. (a) When the $\varepsilon$ of the solvent is not fixed, $C_c$ of both MgCl$_2$ (solid triangles) and CaCl$_2$ (solid circles) decreases with increasing temperature. $\varepsilon$ (solid squares) drops as temperature increases. (b) $C_c$ of MgCl$_2$ (circles) decreases with the amount of ethanol at 20 °C. Addition of ethanol leads to decrease of $\varepsilon$ (squares).
FIG. 2: Effect of temperature on $C_c$ under fixed dielectric constants and fixed $l_B$. At $\varepsilon = 73.2$ (circles) and $\varepsilon = 69.9$ (squares), $C_c$ increases with increasing temperature. When the solution dielectric constant is adjusted to fix $l_B$, $C_c$ doesn’t change with temperature. At fixed Bjerrum lengths, $C_c$ is 45 mM with $l_B = 7.28$ (up triangles), and 40 mM with $l_B = 7.38$ (down triangles).
FIG. 3: $C_c$ as a function of $l_B$. Results for MgCl$_2$ (filled triangles) and CaCl$_2$ (filled circles) indicate that $C_c$ decreases exponentially with increasing $l_B$. Dashed lines are results of exponential fittings to the experimental results.