Counterion Association and Structural Conformation Change of Charged PAMAM Dendrimer in Aqueous Solutions Revealed by Small Angle Neutron Scattering

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Summary: Our previous study of the structure change of poly(amidoamine) starburst dendrimers (PAMAM) dendrimer of generation 5 (G5) have demonstrated that although the overall molecular size is practically unaffected by increasing DCl concentration, a configurational transformation, from a diffusive density profile to a more uniform density distribution, is clearly observed. In the current paper, the focus is placed on understanding the effect of counterion identity on the inter-molecular structure and the conformational properties by studying the effect due to DBr using small angle neutron scattering (SANS) and integral equation theory. While the overall molecular size is found to be essentially unaffected by the change in the pD of solutions, it is surprising that the intra-molecular configurational transformation is not observed when DBr is used. The overall effective charge of a dendrimer is nearly the same for $\alpha < 1$, independent of the type of acids. However, when $\alpha > 1$, the effect of counterion identity becomes significant, the effective charge carried by a charged G5 PAPAM protonated by DBr becomes smaller than that of solutions with DCl. As a consequence, a counterion identity dependence of counterion association is revealed: Under the same level of molecular protonation, the *specific counterion association*, which is defined as the ratio of bound chloride anions to positively charged amines per molecule, is larger for the G5 PAMAM dendrimer charged by DBr than the one by DCl.

Keywords: counterion; ornstein-zernike equation; polyamidoamine dendrimers; small angle neutron scattering

Introduction

Dendritic polymers, or dendrimers, are a new class of macromolecules which can be synthesized by the repetitive addition of a branching unit onto a central core molecule. Each grafted layer is defined as a new generation. With a tree like well-defined architecture but accessible interior, they are distinguished from linear polymers and traditional colloids. This unique intra-molecular structure gives them a rich phase behavior.[1] Consequently, study dendrimer has been a fashion in soft condensed matter research. In addition to the fundamental scientific interest, dendrimer science is also driven by the great potential in their

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applications in several industrial fields including catalysis, medicine and oil. As a result, poly(amidoamine) starburst dendrimers (PAMAM) with ethylenediamine core are the most widely studied ones among more than fifty families of dendrimers. With the presence of primary amines as the terminal groups and tertiary amines locating at the branching points, they can be ionized via the protonation of amines by acidifying the solutions when dissolved in aqueous solutions.

In practical applications, such as drug delivery scaffolds and chelating agents, ionic dendrimers are designed to operate at different aqueous environments with biological and environmental relevant pH value as guest transport vehicles.[2] To facilitate their use in these guest-host applications, a clear understanding of the ionic binding between the different guest electrolytes and the charged constituent components of dendrimer is necessary.

To address this critical issue, we present a systematic study aiming to explore the effect of the identity of anion in an aqueous environment with changing acidity, based on our previously developed experimental approach combining small-angle neutron scattering (SANS) along with a model developed from standard liquid theory,[3] which has been successfully used for investigating the structural properties of charged PAMAM dendrimers and quantitatively revealed the evolution of the intra-dendrimer structure and inter-dendrimer interaction as a function of molecular protonation. Furthermore, we also have observed a generational dependence of chloride association with different degree of molecular protonation.[4]

In this study, our research focus is placed on investigating the difference in counterion association of the chosen monovalent anions, chlorides and bromides respectively, in generation 5 (G5) PAMAM dendrimer. Through analyzing the SANS patterns of the charged PAMAM solutions, the average number of associated counterion is uniquely determined from the effective charge and the ionic strength extracted from the mean-field model.

Materials and Small Angle Neutron Scattering (SANS)

The G5 PAMAM dendrimers used in this work were purchased from Dendritech Inc., Midland, MI, USA.[5] Deuterium chloride, deuterium bromide and deuterium oxide were obtained from Cambridge Isotope Laboratories, Inc., Andover, MA, USA.[5] The preparation of the samples studied in this investigation is detailed in a separate reference.[3] The total amount of amines contained in each sample is kept at a constant value by fixing the dendrimer concentration at 0.0225 g/ml. The METTLER TOLEDO S20 SevenEasy™ pH meter[5] was used to extract the pD, which is converted by the reading of the pH meter according to a generally accepted relation $pD = pH + 0.41$.[3] Furthermore, the deuteron concentration is corrected from the measured deuteron activity.[6]

The concentration of acid added is represented by the acidity scale factor α which is defined as the molar ratio of acid to primary amine terminal groups:

$$\alpha \equiv \frac{[\text{Acid}]}{[-\text{NH}_2]} = \frac{[\text{Acid}]}{2^{n+2}[\text{PAMAM}]} \quad (1)$$

where n is the dendrimer generation

The samples are prepared with different α values ranging from 0 to 1.8. The SANS measurements were carried out using the NG3 SANS spectrometer at the NCNR NIST. The wavelength of the incident neutron beam was chosen to be 6.0 Å, with a wavelength spread $\frac{\Delta\lambda}{\lambda}$ of 15%, to cover values of the scattering wave vector Q ranging from 0.0045 to 0.45 Å^{-1}. The measured intensity $I_{\text{exp}}(Q)$ was corrected for detector background and sensitivity and for the scattering contribution from the empty cell and placed on an absolute scale using a direct beam measurement.[7] All the experiments were carried out at a controlled temperature of $23.0\,^{\circ}\text{C} \pm 0.1\,^{\circ}\text{C}$.

In this study, $I_{exp}(Q)$ is modeled by the following integral equation

$$I_{exp}(Q) = \int_0^{\infty} \frac{I_{model}(z)}{\sqrt{2\pi\delta(Q)^2}} \exp\left[-\frac{(z-Q_m)^2}{2\delta(Q)^2}\right] dz \quad (2)$$

where $I_{model}(Q)$ is the theoretical intensity distribution, $\delta(Q)$ the width of the resolution function at Q and Q_m is the mean Q value.[7] We only briefly outline the major elements of $I_{model}(Q)$ with details reported in Reference [3].

The factorization approximation is used to calculate $I_{model}(Q)$ in this study:[8] The coherent scattering is in principle proportional to the scattering contrast between dendrimers particle and the solvent, the normalized intra-dendrimer structure factor $P(Q)$, and the inter-dendrimer structure factor $S(Q)$. $P(Q)$ is given by the modified fuzzy ball model which approximates the PAMAM molecular density profile by the convolution of a hard sphere with radius R and a Gaussian with variance σ^2 characterizing the soft shell region;[3] and $S(Q)$ is obtained by numerically solving the Ornstein-Zernike integral equation (OZ) for one component system in combination with the hypernetted chain (HNC) closure.[9] The effective inter-dendrimer interaction is approximated by the screened Coulombic interaction potential and the essential physical quantities characterizing the charged PAMAM solutions such as the effective charge carried by a single PAMAM dendrimer and the ionic strength are converted from the fitted potential parameters based on the generalized one-component macroion approach (GOCM).[10]

Results and Discussion

In Figure 1, the neutron scattering intensity distribution as function of transferred wave vector, Q, for G5 PAMAM solutions at $\alpha = 0$ (Panel a), $\alpha = 1.6$ acidified by DCl (Panel b) and $\alpha = 1.6$ acidified by DBr (Panel c), are presented to demonstrate the qualitative structural features revealed by SANS experiment. With the concentration of 0.0225 g/ml, the nearly non-interacting nature of neutral PAMAM dendrimers in solution is reflected clearly by the Guinier region, namely the flat plateau, observed when $Q < 0.03$ Å^{-1} in the SANS pattern presented in Panel a. Upon acidifying the dendrimer solutions, the establishment of the inter-PAMAM screened Coulombic repulsion, as a consequence of the protonation of amines, is reflected by the appearance of the interaction peak cantered at 0.05 Å^{-1}, as the examples showed in Panels b and c.

Although the SANS spectra presented in Panels b and c are seen to be nearly identical in the logarithmic scale, a quantitative

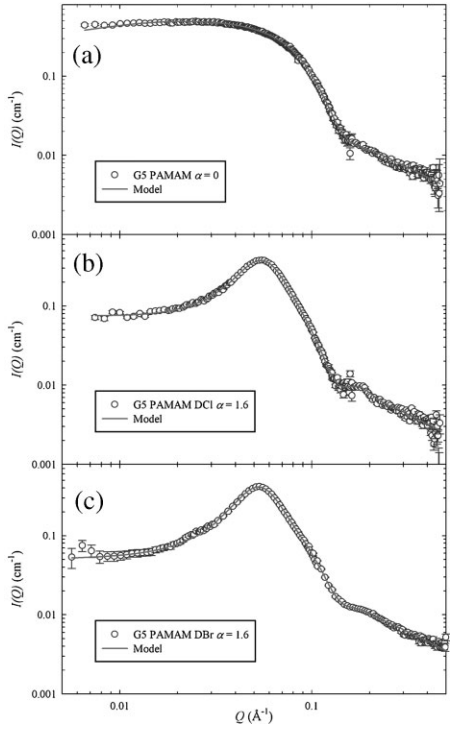

Figure 1.
SANS spectra selected from the G5 PAMAM dendrimers and their charged states in D_2O solutions with a fixed dendrimer weight fraction of 0.0225 g/ml for (a) $\alpha = 0$, (b) $\alpha = 1.6$ which the solution is protonated by DCl and (c) same as (b) but protonated by DBr.

difference is surely discernable: 1) The effective charge for a dendrimer shows the dependence on the type of acid and is about 35 and 28 in DCl and DBr solutions respectively at $\alpha = 1.6$. 2) Upon increasing the molecular protonation by progressive introduction of DCl to the solutions, a gradual formation of a small bump (~ 0.17 Å^{-1}) as shown in Panel b is clearly observed, which reflects the intra-molecular conformation change from a diffusive density profile to a more uniform density distribution.

However, this feature is much less pronounced in the SANS pattern corresponding to the charged PAMAM solutions acidified by DBr, as an example given in Panel c.

The effect of protonation on the molecular conformation, which is characterized by the radius of gyration R_G extracted from our model fitting, is shown in the top panel of Figure 2. For G5 charged PAMAM solutions acidified by either DCl or DBr, R_G is found to be around 25 Å with only a minor molecular swelling, reflected by the $<5\%$ increase of R_G, within the studied pD range. This observed invariance of R_G is intrinsically consistent with the result of a latest computational study carried out by Buzza et al. which concludes the independence of dendrimer conformation on the protonation of amines due to the offset of the inter-charged amine repulsion in PAMAM dendrimer by the local charge neutrality.[11]

Although the dependence of the molecular conformation of G5 PAMAM dendrimers on α, protonated either by DCl or DBr, is characterized by a nearly constant R_G, a clear difference in the dependence of the intra-molecular structure on the counterion introduced by added acid is found: As indicated by the inset in the top panel of Figure 2, for the case of DCl, within the α range studied in this report, the hard-core radius R increases gradually. Meanwhile, σ, the parameter used to quantify the dendrimer molecular diffusive periphery, keeps decreasing upon increasing α. However, unlike the case of DCl, our model

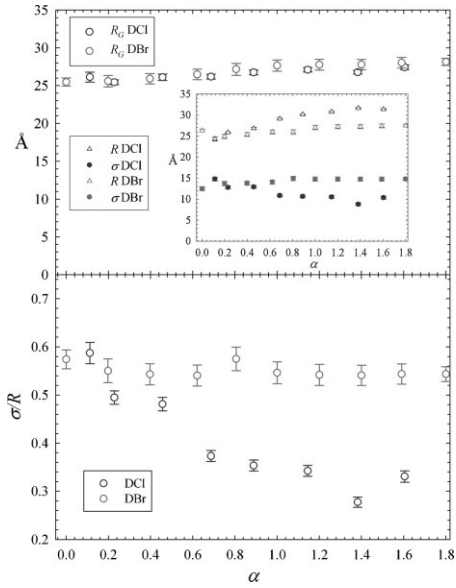

Figure 2.

Top Panel: Evolution of the radius of gyration R_G as a function of α. A weak dependence on molecular protonation is observed. The inset gives the dependence of intra-dendrimer structure factor $P(Q)$ parameters R and σ on α. **Bottom Panel** gives the dependence of the ratio of σ to R as a function of α. A configurational transformation from a diffusive density profile to a more uniform picture is observed when the PAMAM molecule is charged by DCl. However, an invariance in molecular configuration is seen when the samples are acidified by DBr.

fitting suggests that both R and σ essentially remain unchanged upon the progressive protonation of amines by DBr. This difference can be visualized more clearly by plotting the ratio of σ to R. for both cases, as a function of α as shown in the bottom panel. According to the fuzzy ball picture incorporated in our model via $P(Q)$,[3] protonation of the PAMAM amines by DCl results in a transformation of the intra-molecular density profile from a more diffusive density distribution ($\sigma/R \sim 0.6$) at the neutral state to a more uniform, hard sphere-like configuration ($\sigma/R \sim 0.3$) when the amines are fully charged. We have pointed out in our previous report that experimentally this transformation is manifested by the formation of the high Q bump in the corresponding $I(Q)$. Along with the

observed absence of the marked bump in the high Q region of $I(Q)$ presented in Panel c of Figure 1, this point is reascertained by the relative insensitivity of σ/R on α obtained from the SANS model fitting of the DBr-acidified G5 PAMAM dendrimer solutions displayed in the bottom panel of Figure 2.

From the requirement of overall charge neutrality and assuming that all the deuterons inside the dendrimers are associated with the amines, the total number of protonated amines per G5 PAMAM molecule, namely the structural charge, can be calculated with the knowledge of the deuteron concentration, which is converted from the measured pD value in our work, and the amount of total added chlorides.

From the top panel of Figure 3, upon progressively acidifying the solutions, the molecular protonation is seen to increase linearly within the α range of 0 to approximately 1.8. The difference in structural charge obtained from the solutions acidified by DCl and DBr is not perceptible and this observation suggests that the ionic binding between the deuteron and the amine is not affected by the presence of different anion species.

Due to its openness, each dendrimer carries a large cavity accessible by the solvent molecules and ions.[1,12–13] Therefore, once a dendrimer is charged, a large number of counterions can penetrate inside, which is termed as associated counterions in contrast to the free ions moving around outside of a dendrimer. The number of associated counterions as a function of α is shown in Figure 3. As expected, the average number of associated counterions per charged G5 PAMAM dendrimer increases steadily as the structural charge increases. The association of such a large number of counterions will certainly affect the interactions between components of a dendrimer, which in turn affect the overall conformation change. In addition, a species dependence of counterion condensation is observed: With the same degree of molecular protonation, there are more associated bromide anions

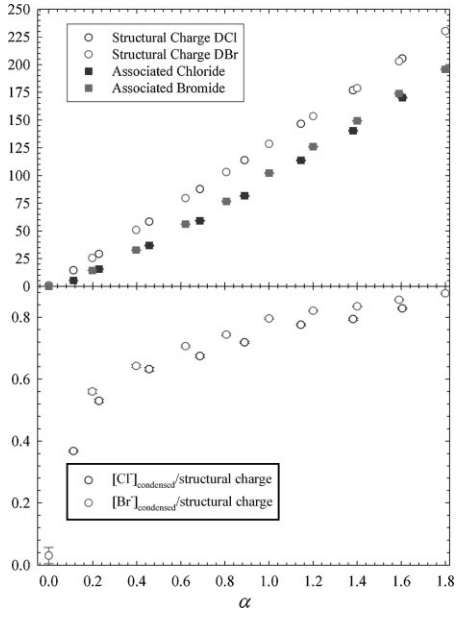

Figure 3.
Top Panel gives the structural charge, namely the total charged amines, and the average of associated counterion as a function of α for G5 PAMAM dendrimers in D_2O solutions. The values are calculated from the macroscopic charge neutrality incorporating the correction of the deuteron activity. Error bars are smaller than the symbol size. **Bottom Panel** presents the specific counterion condensation, defined as the number of the condensed chlorides divided by the number of the charged amines, as a function of α. With the same condition of molecular protonation, the degree of bromide association is seen to be higher than the one of chlorides.

in a dendrimer. The calculated number of condensed counterions per charged amine defined as *specific counterion condensation* is presented in the bottom panel of Figure 3. The condensed counterion density of G5 PAMAM charged by DBr, for a given α, is higher than the one by DCl. This finding is essentially consistent with the higher affinity of bromide, in comparison to chloride, toward the oppositely charged macroions in the solutions which has been repeatedly reported in various charged colloidal systems including proteins, ionic micelles and polyelectrolytes.[14]

There has been extensive effort devoted to providing rational interpretation to this observation which cannot be explained by

the DLVO theory of charged colloid stability. The ion specificity theory, one of many attempts for improving the DLVO theory, to explain the dependence on the identity of the anion: It is proposed by Ninham et al. that,[15] due to the excess polarizability, which is defined as the difference in polarizability of an anion compared to a water molecule with same volume and different from one anion to another, an additional ionic dispersion force is presented in the interaction between the anion and macroions. When this non-electrostatic force is properly incorporated into the DLVO theory, several unaccounted experimental observations related to the salt identity such as ion-specific inter-colloidal interaction can be explained in a systematic and quantitative manner.

It is sensible to attribute the origin of our experimental observation to this ion specific effect, at least to some certain extent. However, it should be noted that the complexity of ionic binding in charged PAMAM dendrimer is compounded by the structural openness at molecular level. As indicated by several computational studies, a considerable difference in density of the water buried inside the internal cavities and bulk water well outside the dendrimer is found due to the confinement effect. This confinement effect may thus affect the mobility of associated counterions and other solvent molecules and eventually affect their interactions with the dendrimer. Qualitatively, it is plausible that, the difference in overall counterion packing shown in Figure 3 and the inter-molecular density profile shown in Figures 1 and 2 may be the manifestation of the modification of the spatial distribution of counterion, due to the change in the magnitude of interaction potential. However, it is evident to us that, to provide the detailed spatial counterion distribution within charged dendrimer molecule at microscopic level, further information provided by other comparative experimental means or theoretical calculations are necessary to complement the conclusions of our mean-field SANS experimental approach.

The effective charge carried by a single dendrimer molecule is determined by the difference between the number of charged amines and the number of associated counterions. This value is directly extracted from the SANS intensity fit, which is set by a balance between electrostatic interactions, which favor counterion condensation, and entropic effects, which prohibit complete counterion condensation. The dependence of effective charge on α, obtained from both samples acidified by DCl and DBr, is characterized by a similar feature: As shown in Figure 4, at low α values ($\alpha < 1$), where the molecular protonation is contributed entirely by charged primary amines, the effective charge is seen to increase nearly linearly. At larger values of α ($\alpha > 1$) at which the tertiary amines start to be protonated, the effective charge shows only a very weak dependence on α. Especially, when $\alpha < 0.8$, there is essentially no difference between the effective charge for a dendrimer acidified by either DCl or DBr. However, when $\alpha > 0.6$, the effective charge shows a dependence on counterion identity, with dendrimers protonated by DCl carrying a higher effective charge. The effective charge of a G5 dendrimer saturates at about 25 for DBr and 35 DCl

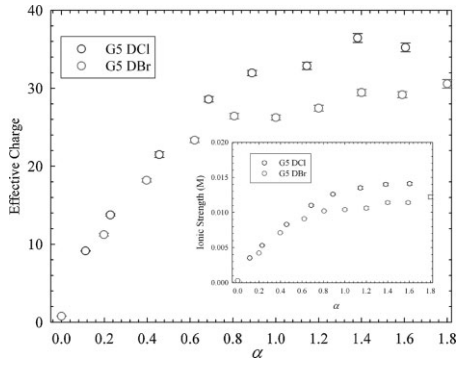

Figure 4.
The effective charges carried by a dendrimer molecule obtained from the SANS model fittings as a function of α. Due to the higher affinity of bromides to the charged dendrimer, the effective charge obtained from the G5 PAMAM solutions acidified by DBr is smaller than the one for the solutions acidified by DCl.

respectively. In our previous report, the invariance of the effective charge at $\alpha > 1$ is attributed to the localization of counterions enhanced by the open molecular architecture. The ionic strength of the medium as a function of α is given in the inset. The observed lower ionic strength in the medium of G5 dendrimer samples with DBr is again consistent with the higher affinity of bromide toward the charged PAMAM molecules.

Conclusions

We carried out a SANS study of charged G5 PAMAM aqueous solutions focusing on understanding the effect of counterion identity on the molecular conformation and inter-dendrimer interaction. No significant change in R_G, commonly used in describing the dendrimer molecular size, upon protonation of the G5 dendrimer, either by DCl or DBr. However, a discernable change in the intra-molecular density profile from a diffuse structure at neutral pD to a hard-sphere like conformation is clearly seen for the G5 PAMAM dendrimer charged by DCl.

Furthermore, our SANS model fitting also reveals a dependence of counterion condensation on the identity of counterion: Under the same degree of molecular protonation, bromides are found to show a higher affinity toward the charged G5 PAMAM dendrimers in comparison to chlorides. It is our hypothesis that our observation of the dependence of molecular configuration and counterion association on counterion identity may have some certain connection with the ion specificity.

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[15] See V. Deniz, M. Boström, D. Bratko, F. W. Tavares, B. W. Ninham, *Colloids and Surfaces A: Physicochem. Eng. Aspects* **2008**, *319*, 98 and references therein.

