Cooperativity in Macromolecular Interactions as a Proximity Effect: NMR and Theoretical Study of Electrostatic Coupling of Weakly Charged Complementary Polyions

Jaroslav Kříž,* Jiří Dybal, and Dana Kurková

Institute of Macromolecular Chemistry, Academy of Sciences of the Czech Republic, Heyrovsky Square 2, 162 06 Prague 6, Czech Republic

Polyelectrolyte behavior and cooperativity of electrostatic coupling of two weakly charged polyions, poly-(sodium methacrylate)-*co*-(acrylamide) containing 10 mol % ionic groups (A10) and poly(*N*-diallyldimethylammonium chloride)-*co*-(acrylamide) containing 8 mol % ionic groups (C8), was examined using ^{1}H, ^{23}Na, and [35]Cl NMR and ^{1}H PGSE NMR, ab initio SCF HF/6-31G(d) calculations, and simulations using a simplified theoretical model of *insular* equilibria. With the use of ^{23}Na and [35]Cl NMR relaxations, it was first shown that neither A10 nor C8 exhibits polyelectrolyte effects in the sense of counterion condensation in dilute aqueous solution. Accordingly, electrostatic binding of low-molecular-weight models of the charged groups, namely sodium pivalate (for A10) and tetramethylammonium chloride (for C8), to the complementary polyion is weakly populated and exhibits no cooperativity (as shown by ^{23}Na and [35]Cl NMR relaxations and ^{1}H PGSE NMR). However, interaction of A10 with C8 in dilute solutions is distinctly cooperative and leads to relatively high coupling degrees. A simplified theoretical model based on a restriction imposed on the motion of the coupling groups by neighboring already coupled pairs, which we present here, explains this behavior and predicts experimental data in a semiquantitative way. The model can be improved by including a detailed description of motional constraints.

Introduction

For many years, cooperativity of interactions has been recognized as an important factor in molecular self-assembly of various functional systems of macromolecules both in nature and in synthetic materials. It has been understood mainly as an additive effect of many individual interactions acting simultaneously and thus leading to an exponentially increasing binding.

However, many actual systems defy such a clearcut explanation. The case in point is electrostatic interaction of complementary polyions. Electrically charged macromolecules such as polycations and polyanions are known to produce macromolecular complexes primarily based on long-range electrostatic interactions of opposite charges[1−19] (for a fuller overview of the field, see in particular refs 1−3 and references therein). According to the structure of polyions used and conditions of their mixing, different complex formations starting from molecular complexes up to nanoparticles, physical networks, and gels can be produced. The underlying interaction between complementary polyions has been shown to be cooperative.[20−23] However, the driving force of this effect appears to be mostly an entropy gain achieved by the liberated small counterions and hydrating water molecules (although hydrophobic interaction could also play a role in some cases[23]). In the case of densely charged polyelectrolytes, this effect is clearly due to the well-known counterion condensation, i.e., their preferential distribution in the vicinity of the original polyions, which is energetically favorable but low in entropy. Hence the counterions, liberated by polyion coupling, achieve much higher entropy due to their random distribution in the whole volume. Accordingly, increased charge density and—to some extent—chain length of the polyions, which lead to the correlated increase in counterion

condensation, have an observed effect of increased cooperativity in polyion coupling.

This explanation cannot be considered to be quite universal, however. Cooperativity, albeit weaker, has been observed in the couplings of weakly charged polyions (e.g., random copolymers of ionic and electrically neutral monomers) with almost no counterion condensation. Additional proposed factors[23] such as hydrophobic interaction and pseudophase effects cannot fully explain cooperativity in these cases.

There is, however, another effect common to all polyvalent molecules including weakly charged polyions, where specific polyelectrolyte effects are virtually absent and random statistics of positions, motions, and collisions could be assumed. We shall call it "insular kinetics" for reasons that shall become apparent immediately.

Consider a collection of lone groups a and b that are capable of equilibrium coupling to a product (ab), with the equilibrium degree of their coupling at the given concentration being α. Now, assume that each *m* of groups a are interconnected into a linear string by completely neutral and flexible links of some length and so are the groups b. By neutrality of the links we mean their complete incapability of specific interaction either with groups a and b or with each other. Let us also assume that the links are long enough so that neither of the linked groups influences each other directly or indirectly (e.g., by causing counterion condensation due to their integrated electric field in the case of polyions). Under such conditions, no existing model (according to our knowledge) predicts any cooperativity in the coupling reactivity of a or b: the groups should behave exactly as if they are free; i.e., they should couple to the same degree α.

However, this appears to be so only if we ignore the effect that the interconnectedness of groups (a or b) in a string has on

* Corresponding author. Phone: 420-296809382. Fax: 420-296809410. E-mail: kriz@imc.cas.cz.

the probability of encounter of the complementary groups. The probability of a first successful encounter of a pair of groups a and b is given purely by their respective probability densities (i.e., concentrations or activities) and their translation mobilities and so is, from the statistical point of view, the probability of their reverse parting. However, the conditional probability of encounter of another pair of groups of the same strings is changed as the strings are already bound to each other. In contrast to the former case, where the respective groups had access to the whole volume of the system, the first link strongly restricts the effective volume V_{ef}, in which the yet uncoupled groups of the bound strings can move. This statistical distortion increases as the effective volume shrinks with each additional link. The motional restriction results in a marked change of coupling rate (and, consequently, of the respective equilibrium constant), which manifests itself as a cooperative effect.

Macromolecules with reactive groups interleaved by neutral segments can serve as examples of such strings. Consider two macromolecules A and B, each bearing a number of respective complementary interacting groups (such as opposite charges) $a_1, a_2, ..., a_m$ and $b_1, b_2, ..., b_n$ in a dilute solution. Let us assume that the free activation energy of coupling is the same for any couple $(a_p b_q)$, so that the kinetics will be given purely by the statistics of collisions. The restriction of relative motions of a and b in an already partly coupled complex A·B can be translated into *effective concentrations* $[a]_{ef}$ and $[b]_{ef}$. If $[a]_{ef} \gg [a]$ (and, accordingly, $[b]_{ef} \gg [b]$), the couplings inside the given complex A·B are much more probable than those of A·B with another A or B. This happens in dilute solutions and is utilized intuitively by synthetic chemists in preparation of, e.g., cyclic compounds. However, the same effect leads to a dramatic shift of *equilibria* in a manner quite analogous to cooperative effects. There are two reasons for this: (i) the concentration dependence of the rate of a reversible reaction a + b ↔ (ab) is quadratic from left to right but only linear in the opposite direction; (ii) the *effective concentrations* $[a]_{ef}$ and $[b]_{ef}$ increase by previous coupling of the macromolecules whereas that of the coupled pair $[(ab)]_{ef}$ does not. Again, this effect is familiar in physical organic chemistry where, e.g., equilibria of intramolecular hydrogen bonds are strongly shifted by proximity of the respective bound groups.

The quantitative model slightly changes under the assumption that more than two macromolecules can interact to form a product. However, the principle remains the same. We have to bear in mind that the product of *n*-valent macromolecules forms a more or less dense network, which can absorb only a limited amount of solvent (according to its limiting swelling volume). In a very dilute solution, the product thus always forms supermolecular islands surrounded by a much larger volume of solvent. On these grounds, we could perhaps call the kinetics and equilibria in such systems *insular*.

In this paper, we examine both a simplified theoretical model of the insular equilibria and compare it with experimental results obtained for weakly charged polyions based on sodium methacrylate (MANa) and *N*-diallyldimethylammonium chloride (DADMAC). The logic of the paper is the following. First, we demonstrate that our weakly charged copolymers exhibit no counterion condensation (polyelectrolyte effect), which could cause their cooperative coupling based on the entropy change of liberated counterions, and behave like a collection of lone charged groups from the electrostatic point of view. Second, we select low-molecular-weight models of these groups and demonstrate that their charge distribution copies that of the groups in the polymers. Third, we establish the equilibrium

constants of coupling of these models to the complementary groups in the polymers. Fourth, we demonstrate that the interaction of two complementary polymers under comparable conditions results in a coupling degree that is at least 1 order of magnitude larger than that expected for lone groups. Finally, we compare these results with our simplified model and show that its prediction is semiquantitatively right.

Experimental Section

Materials Used. PDADMAC ($\overline{M}_n = 1.6 \times 10^5$, $Z = 898$) and the statistical copolymer of 8 mol % DADMAC with 92 mol % acrylamide (C8, $\overline{M}_n = 5.15 \times 10^5$, $Z = 597$) were prepared[24] by radical polymerization of the respective monomers. PMANa ($\overline{M}_n = 1.58 \times 10^5$, $Z = 1458$) was purchased from Polymer Source, Inc. Statistical copolymer of 10 mol % MANa with 90 mol % acrylamide (A10, $\overline{M}_n = 3.18 \times 10^5$, $Z = 425$) was prepared[25] by radical copolymerization of the respective monomers. Sodium trimethyl acetate (TMANa) and *N*-tetramethylammonium chloride (TMAC) were purchased from Aldrich in laboratory grade. D_2O (99.9% of ^2H) was purchased from Chemotrade Chemiehandelsgesellschaft mbH, Germany.

Preparation of Samples. In a typical example, $\beta \times 1.0$ mL (β in the range 0.1−0.6) of a solution of a polyanion ($[A]_0 = (2.0−10.0) \times 10^{-3}$ mol/L) in D_2O was added at a rate of 0.2 mL/h to a vigorously magnetically stirred (250 rps) mixture of 1.0 mL of a polycation ($[C]_0 = [A]_0$) and $(1 - \beta) \times 1.0$ mL of D_2O using a Genie syringe pump. A 0.6 mL volume of the sample was transferred into a 5 mm NMR tube.

NMR Measurements. ^1H, ^{23}Na, and ^{35}Cl NMR spectra, relaxations, and pulsed-field-gradient spin−echo (PGSE) measurements were obtained using a Bruker Avance DPX300 instrument with a broad-band inverse-detection probe head (proton spectra, relaxations, and part of PGSE), broad-band direct-detection probe head (^{23}Na and ^{35}Cl spectra and relaxations), and a water-cooled inverse-detection diffusion probe head with an additional PFG unit (gradients up to 1000 G/cm). Most of the methods used were described earlier.[20−23,31] ^1H PGSE or PFG-SSE (pulsed-field-gradient stimulated echo) were done using the simple Tanner−Stejskal or Tanner sequences with 1 ms pulses of field gradients incremented in the range 15−50 G/cm (for low-molecular-weight models) or 80−720 G/cm (for macromolecular complexes) and constant diffusion delay (typically 200 and 50 ms, respectively).

Quantum Mechanical Calculations. The ab initio SCF calculations were done in the HF/6-31G(d) format using the Gaussian program.[26] The structures were optimized to energy minima without geometrical or other constraints.

Theoretical Model

Let us first consider a simple equilibrium between reactants p and q and their addition product r:

$$p + q \rightleftharpoons r$$
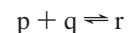
As is generally known (see, e.g., ref 32), the total numbers of molecules N_p, N_q, and N_r in the equilibrium (approximating the system as an ideal gas) obey the relation

$$\frac{N_r}{N_p N_q} = \frac{z_r}{z_p z_q} e^{-\Delta E_0/kT} \tag{1}$$

where ΔE_0 is the energy difference between the ground states of the reactants and the product. z_x is the partition function (pf)

SCHEME 1
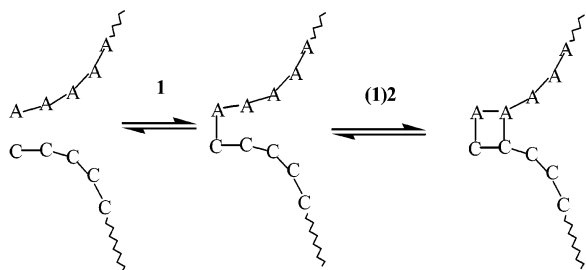
of the component x, which is a product of translation pf (tpf) z_{tx} and internal (rotation, vibration, and electronic) pf: $z_x = z_{tx}z_{ix}$. Whereas z_{ix} values are dependent on temperature only, z_{tx} depends on the volume V, too: $z_{tx} = (2\pi m_x kT/h^2)^{3/2}V$. For independent molecules, volume V is the same for all components, so that one can write

$$\frac{N_r/V}{(N_p/V)(N_q/V)} = \frac{\rho_r}{\rho_p\rho_q} = K(T) = \frac{z_r'}{z_p'z_q'} e^{-\Delta E_0/kT} \quad (2)$$

where $z_x' = z_x/V$ and ρ_x is the population density (number of molecules per volume unit) of the species x. Assume now that pairs of molecules of p and q are restricted in their relative motion to some small volumes v. Let us state for simplicity that all p's and q's are paired in such a way and the number of pairs is $N_{pq} = N_p = N_q$. Then we have, in analogy to eq 1

$$\frac{N_r}{N_{pq}} = \frac{z_r}{z_{pq}} e^{-\Delta E_0/kT} \quad (3)$$

where z_{pq} is the pf of the pair pq. If molecules p and q in the pair pq are not restricted in their internal degrees of freedom, we can write $z_{pq} = z_t z_{ip} z_{iq}$, where the first term is the translation pf (tpf) of the pair and the other two are internal pf's of p and q. Assuming now that the translation of the pair pq and the relative translation of p and q can be separated (which is a rather gross approximation for small molecules but will be better satisfied below), we can approximate $z_t = z_{tpq}z_{ti}$, where the first term is tpf of the center of gravity of the pair, $z_{tpq} = [2\pi(m_p + m_q)kT/h^2]^{3/2}V$ and the second one is the internal tpf $z_{ti} = (2\pi\bar{m}_{pq}kT/h^2)^{3/2}v$, with the reduced mass $\bar{m}_{pq} = m_p m_q/(m_p + m_q)$. It is easy to see that

$$\frac{(N_r/V)}{(N_{pq}/V)} = \frac{\rho_r}{\rho_{pq}} = K(T)/v \quad (4)$$

Transforming ρ_x into molar concentrations [x], eq 2 reads $[r] = K(T)[p]N_A[q]$, where $N_A \approx 6 \times 10^{23}$, and eq 4 reads $[r] = K(T)[pq]/v$. Relative to the unrestricted case, restriction of relative motion of the reactants thus shifts the equilibrium more to the product if $N_A[q]v < 1$. As an example, assume that the volume to which the (pq) pair is restricted is a sphere with a diameter of 2.5 nm and $[q] = 1.0$ mmol/L; $N_A[q] \approx 6 \times 10^{20}$ L^{-1}, whereas $1/v = 1.22 \times 10^{23}$ L^{-1}, i.e., about 200 times higher.

Consider now an equilibrium interaction of two macromolecules with complementary reactive groups A and C (such as anionic and cationic groups) interleaved by a number m_A and m_C of neutral units, respectively, as shown in Scheme 1. In the absence of any special (such as polyelectrolyte) effects, a primary equilibrium bonding (shown here for the first unit) should be quite analogous to that of low-molecular-weight models of A and B.

Let the corresponding equilibrium constant be K (in mol/L). However, the nearest next bonding (symbolized as (1) 2) is formed under restricted relative motion of the second pair of A and C. In an intentionally simplified model, the groups are allowed to translate freely in a spherical volume with a diameter given by the largest possible distance of groups A and C in this pair, which is $m_A + m_C$. The restricted volume thus is $v = \pi(m_A + m_C)^3 d^3/6$, where d is the distance across one interleaving unit (the actual restriction will of course be higher and more complicated in any real case).

For further analysis of bonding, any product of the two macromolecules can be uniquely represented by an $n_A \times n_C$ matrix **P** (where n_A and n_C are the numbers of groups A and C, respectively), with the element $P_{ij} = 1$, if the ith group A with the jth group C are bound, and zero otherwise. Note that only one element in each row or column can be 1. It is easy to show that matrices with "antidiagonal" motifs are extremely improbable on steric grounds and need not be considered. If strong proximity effects (in the sense indicated above) can be expected, it is easy to see that the most probable products are those with consecutive bonds, i.e., represented by matrices, which are either diagonal or have nonzero elements parallel to the main diagonal. Even then, a rigorous description of the equilibrium system could be very complicated. At present, we illustrate the basic idea of our approach using a number of simplifying assumptions.

We shall name $\mathbf{P}_r^s$ the product with r consecutive bonds, the last being placed at the sth place, i.e., represented by a diagonal matrix with $P_{s-r,s-r} = P_{s-r+1,s-r+1} = ... = P_{s,s} = 1$. For simplicity, let us start with $r = s$. Then we have

$$[\mathbf{P}_1^1] = K[A][C]/(n_A n_C) \quad (5)$$

$$[\mathbf{P}_r^r] = K_i\xi[\mathbf{P}_{r-1}^{r-1}] = KK_i^{r-1}\xi^{r-1}[A][C]/(n_A n_C) \quad (6)$$

where [A] and [C] are molar concentrations of A and C groups, K_i is the internal equilibrium constant, and, with the present definition of K, $\xi = 1/(N_A[A]_0 v)$. Let be $n_A \ll n_C$ and the ratio $\beta \approx [A]_0/[C]_0 \leq 1$. Let α be the degree of binding; $\alpha = 1 - [A]/[A]_0$. Then (taking into account that any group A or C can be the site of the first bond)

$$\alpha = \frac{n_A n_C \sum_{k=1}^{n_A} k[\mathbf{P}_k^k]}{[A]_0} = K[A]_0(1-\alpha)(1/\beta - \alpha)\sum_{k=1}^{n_A} kK_i^{k-1}\xi^{k-1} \quad (7)$$

and naming

$$\psi = K[A]_0\left(\frac{1 + (n_A\xi K_i - n_A - 1)(\xi K_i)^{n_A}}{(1 - \xi K_i)^2}\right) \quad (8)$$

we get

$$\alpha = \frac{\psi(1 + \beta^{-1}) + 1 - \sqrt{[\psi(1 + \beta^{-1}) + 1]^2 - 4\psi^2\beta^{-1}}}{2\psi} \quad (9)$$

Note that for independent groups A and C the expression for α is identical with eq 9, only $\psi = K[A]_0$ in that case. Figure 1 shows some examples of simulation of α using eqs 8 and 9 ($K = K_i = 10$ mol^{-1}, $[C]_0 = 10^{-3}$ mol/L, β is given in the figure). As one can see, pseudocooperativity (i.e., large α) can be achieved if ξ reaches values over 10.

Results and Discussion

Characterization of the Polyions. To be able to discern various kinds of cooperativity in the electrostatic interactions
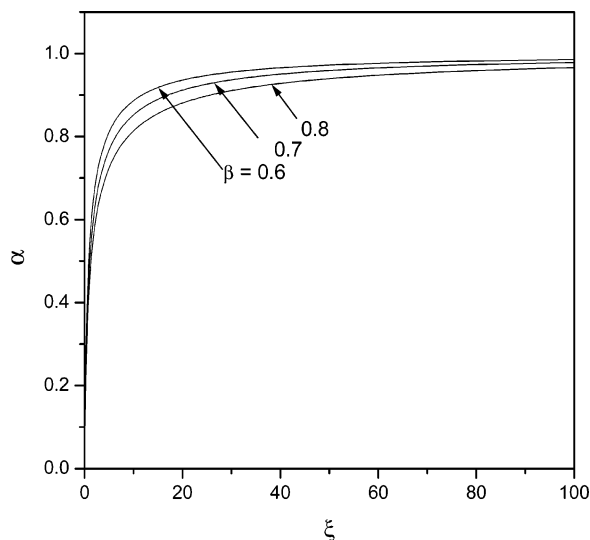
Figure 1. Simulated values of coupling degree α at varying proximity factor ξ and indicated values of $\beta = [A]_0/[C]_0$.

of polyions, we have to eliminate or at least separate specific polyelectrolyte effects. As already explained in the Introduction, the salient point is counterion condensation present in dilute solutions of some polyions. The degree to which the spatial counterion distribution is perturbed in this sense depends on the effective charge density of the polyions (given by both their chemical structure and conformation) and the dielectric state of the system. Probably the most reliable way to test it is provided by quadrupolar relaxation of the counterions. In the present study, we compare the densely charged homopolymers poly(*N*-diallyldimethylammonium chloride) (PDADMAC) and sodium polymethacrylate (PMANa) with the weakly charged copolymers poly(*N*-diallyldimethylammonium chloride)-*co*-(acrylamide) (containing 8.0 mol % DADMAC, named C8) and poly(sodium methacrylate)-*co*-(acrylamide) (containing 10.3 mol % MANa, named A10).

Both natural counterions, namely Na$^+$ and Cl$^-$, contain $I = 3/2$ nuclei ^{23}Na and ^{35}Cl. Our analysis is mostly based on the model of quadrupolar transverse relaxation of counterions in polyelectrolyte systems suggested by Halle et al.[27,28] The authors treat the polyion as a rigid cylinder with radius a in a cylindrical cell with radius $b = (\pi l n_m)^{-1/2}$, l and n_m being the monomer length and number density, respectively. The most important factor in transverse relaxation of the counterion nuclei ($I = 3/2$) is shown to be diffusion of the counterions across the cell's boundary, from one polyion to another (differently oriented) one. In dilute solutions of densely charged polyions, the correlation function of the residual electric field gradient (efg) of the polyion has a long-time tail and, consequently, the relaxation behavior becomes dominated by the zero-frequency spectral density $J(0)$. In the first approximation, $J(0)$ is shown to depend on the probability P of the counterion being present in the perturbed area $r \in \langle a, a + \delta \rangle$, where δ is supposed to be of the order of the first hydration shell radius of the counterion, and on the rate of its diffusion out of the cell, given by the inverse of the radial correlation time τ_{rad}. The average density distribution of the counterions is based on the mean-field Poisson−Boltzmann equation

$$-\epsilon_0\epsilon_r\frac{1}{r}\frac{d}{dr}\left[r\frac{d\psi(r)}{dr}\right] = \sum_i z_i e n_i(b) \exp\left[-\frac{z_i e \psi(r)}{k_B T}\right] \quad (10)$$

Their translational diffusion is treated accordingly, using the Smoluchowski equation

$$\frac{1}{D}\frac{\partial}{\partial t}f(r,t) = \frac{1}{r}\frac{\partial}{\partial r}\left[\frac{\partial}{\partial r} + \sum_i \frac{z_i e}{k_B T}\frac{d\psi(r)}{dr}\right]f(r,t) \quad (11)$$

where $\psi(r)$ and $f(r,t)$ are the electrostatic potential at r and probability to find a counterion in the interval from r to $r + dr$, respectively. With eqs 1 and 2 solved for the given system, both P and τ_{rad} are obtained. The model defines a relaxation rate difference ΔR_2

$$\Delta R_2 = (2/3)[J'(0) - J'(2\omega_0)] \quad (12)$$

where $J'(0)$ and $J'(2\omega_0)$ are the modified spectral densities at zero and double Larmor frequency (see below), respectively. ΔR_2 can be obtained from a careful analysis of the biexponential decays in transverse (or $\mathbf{T}_1{}^1$)

$$\langle I_x(t)\rangle + i\langle I_y(t)\rangle = [\langle I_x(0)\rangle + i\langle I_y(0)\rangle]e^{-i\omega t}\left\{\frac{3}{5}\exp(-[J'(0) + J'(\omega_0)]t) + \frac{2}{5}\exp(-[J'(\omega_0) + J'(2\omega_0)]t)\right\} \quad (13)$$

and longitudinal (or $\mathbf{T}_0{}^1$) relaxation:

$$\langle I_z(t)\rangle = \langle I_z(0)\rangle\left\{\frac{1}{5}\exp[-2J'(\omega_0)t] + \frac{4}{5}\exp[-2J'(2\omega_0)t]\right\} \quad (14)$$

where $J'(\omega) = (\pi^2\overline{\chi_Q}{}^2/10)J(\omega)$; $\overline{\chi_Q}$ is an effective quadrupolar splitting constant at δ and $J(\omega)$ is the spectral density at ω. According to the Halle−Wennerstrom−Picullel (HWP) model[27,28] as well as experimental evidence, $2J'(\omega_0) = 2J'(2\omega_0) = R_1$, so that eq 14 turns to a monoexponential decay

$$\langle I_z(t)\rangle = \langle I_z(0)\rangle \exp(-R_1 t) \quad (14a)$$

and eq 13 remains biexponential

$$\langle I_x(t)\rangle + i\langle I_y(t)\rangle = [\langle I_x(0)\rangle + i\langle I_y(0)\rangle]e^{-i\omega t}\left\{\frac{3}{5}\exp[-(J(0) + R_1/2)t] + \frac{2}{5}\exp[-R_1 t]\right\} \quad (13a)$$

unless $J(0) = R_1/2$, as approximately happens under conditions of extreme narrowing.

ΔR_2 in eq 12 happens to be equal[29,30] to the relaxation rate R_{23} of the second-order third-rank coherence $\mathbf{T}_{23}{}^3$, which can be measured directly by an appropriate pulse sequence:

$$R_{23} = (2\pi^2/3)[J(0) - J(2\omega_0)] \quad (15)$$

According to the HWP model, ΔR_2 can be approximated:

$$\Delta R_2 = (\pi^2/20)\overline{\chi_Q}{}^2 P^2\tau_{rad}/(1 - S_2) \quad (16)$$

where S_2 is an order parameter (supposed to be zero in dilute solutions). For densely charged polyions, it can be shown by simulations that P remains almost constant (counterion condensation) whereas τ_{rad} sharply increases under dilution. By contrast, weakly charged polyions with lone ionic groups behave much like low-molecular-weight electrolytes; i.e., P decreases and τ_{rad} increases under the same conditions. We thus have a very sensitive criterion of the counterion behavior: under dilution: a sharp increase in ΔR_2 (and R_{23}) indicates counterion

Electrostatic Coupling of Complementary Polyions

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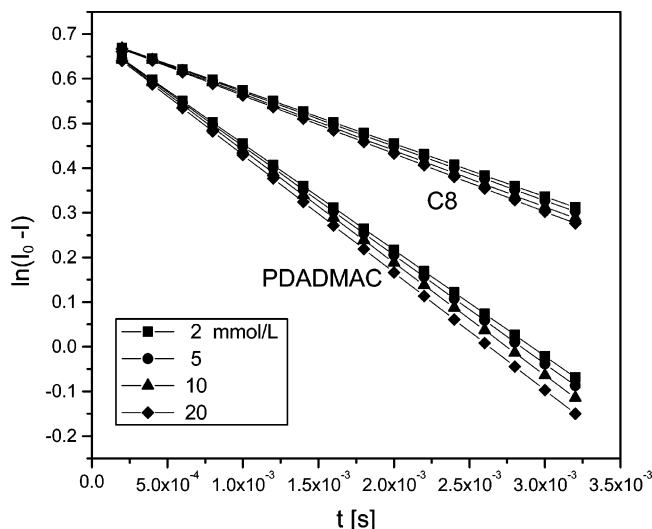

Figure 2. ^{35}Cl NMR longitudinal relaxation decays (semilogarithmic linear fits) in PDADMAC and C8 solutions at the indicated concentration of charged groups (for C8, the curves are in the same order).

condensation (and specific polyelectrolyte effects) whereas a slight decrease or constant (often near zero) value of the same quantities is proof of its absence.

In the case of ^{35}Cl counterions and at concentrations below 10 mmol/L, such analysis is rather involved due to low effective sensitivity of the measurement (2−6 day experiments have to be performed for each decay). Nevertheless, using eqs 13a and 14a makes the analysis possible. Figure 2 gives ^{35}Cl NMR longitudinal decays for PDADMAC and C8 in a semilogarithmic form, showing them to be monoexponential. Thus, R_1 can be obtained without difficulty.

Figure 3a gives ^{35}Cl NMR transverse relaxation decays for the same systems, which were fitted using eq 13a, i.e., assuming the ratio of the two exponentials to be 0.6:0.4 (this ratio being firmly based on the experimentally verified theory of relaxation of spin 3/2 nuclei) and taking R_1 from the previous analysis. Thus, $J'(0)$ could be obtained. The striking difference in relaxation behavior of ^{35}Cl in PDADMAC and C8 is demonstrated in two examples (both 2×10^{-3} mol/L) in Figure 3b with semilogarithmic plots. As is clearly seen, the decay is clearly biexponential for PDADMAC but almost monoexponential for C8.

The ^{23}Na relaxation analysis, which is considerably easier, was done for PMANa and A10 in a quite analogous way. The results of ΔR_2 calculated using eq 12 are collected and compared with the double-quantum ($\mathbf{T}_2{}^3$) relaxation rate R_{23} (in the cases where the double-quantum coherence was indirectly detectable in sufficient intensity) in Figure 4.

As clearly seen in Figure 4, both polyelectrolytes PDADMAC and PMANa exhibit a steep increase in ΔR_2 in the dilute state. As predicted by the HWP model, this increase is due to an exponential increase in the diffusion correlation time τ_{rad}, which is not compensated by a complementary decrease of the probability P (due to counterion condensation). It can be demonstrated (not shown here) that the magnitude of such ΔR_2 effects decreases in both polyelectrolyte types with increasing dilution of the charged groups by neutral AA units in the polymer chain (in contrast to τ_{rad}, the concentration dependence of P changes with charge dilution). They are detectable down to 21 mol % cationic groups in DADMAC−AA copolymers and down to 18 mol % anionic groups in MANa−Na copolymers. However, for both weakly charged polyions C8 and A10 used in this study, there is no such effect: ΔR_2 slightly decreases
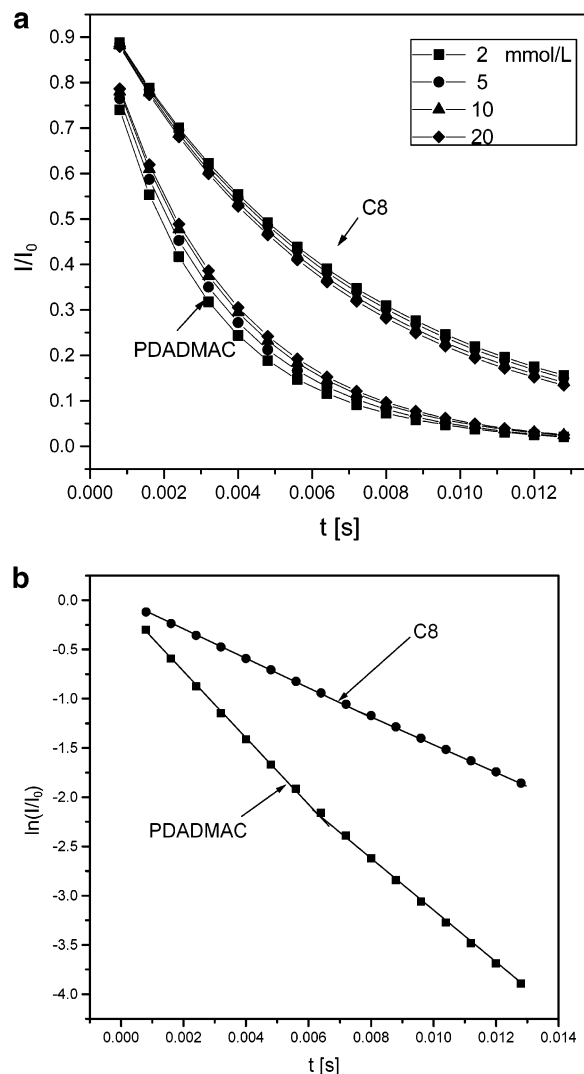

Figure 3. (a) ^{35}Cl NMR transverse $T_1{}^1$ relaxation decays in PDADMAC and C8 solutions at the indicated concentration of charged groups. The biexponential fits are according to eq 13a, the values of R_1 being obtained from the monoexponential fits in Figure 2. (b) Semilogarithmic plots of ^{35}Cl NMR transverse $T_1{}^1$ relaxation decays in PDADMAC and C8 solutions at 2×10^{-3} mol of DADMAC/L.

with dilution as can be expected for polymers devoid of counterion condensation and the correlated effects in their dilute solutions. On the basis of this evidence, we can exclude the entropy gain of the counterions as a driving force of cooperative coupling of C8 with A10.

Electrostatic Binding of Low-Molecular-Weight Models to the Polyions. As both weakly charged polyions C8 and A10 were shown to be virtually free of specific polyelectrolyte effects, their charged groups should behave like being alone, i.e., analogously to low-molecular-weight ions. The next step in our study is an examination of coupling of their low-molecular-weight analogues (or models) with the polyions. As usual, the problem of modeling of chemical groups in a polymer is rather tricky. Scheme 2 compares the structures of the charged groups in question with those we have chosen as low-molecular-weight models, namely sodium trimethyl acetate (TMANa) as model of an MANa unit and tetramethylammonium chloride (TMAC) as model of a DADMAC unit. The corresponding structures are slightly different, but our ab initio SCF quantum mechanical calculations predict quite analogous charge distributions at the relevant atoms of the charged groups as shown in Figure 5. From the point of view of electrostatic interactions,
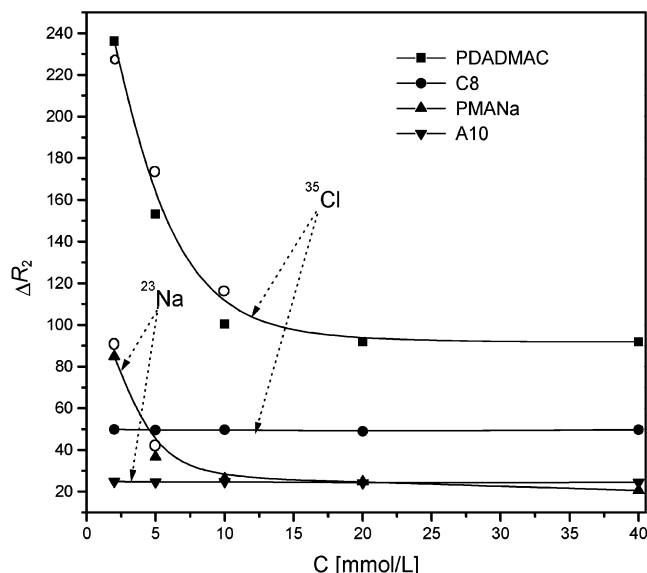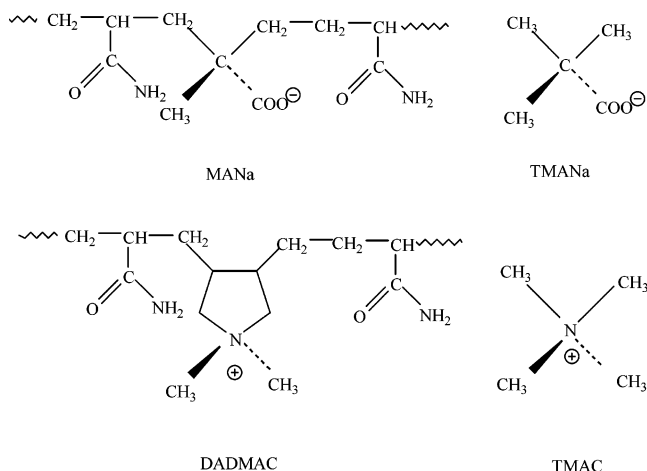
Figure 4. ^{35}Cl and ^{23}Na relaxation increments ΔR_2 for PDADMAC, C8, PMANa, and A10 as a function of concentration of charged groups, obtained from longitudinal and transverse relaxation decays according to eqs 13a and 14a. For PDADMAC and PMANa, independent results obtained from $T_2{}^3$ relaxation are represented by $\bigcirc$.

SCHEME 2

TMANa and TMAC thus can be considered to be comparably good models of the lone charged groups in the polyions A10 and C8, respectively.

In the present study, we examined the degree of binding of TMANa to PDADMAC and C8 and of TMAC to PMANa and A10, respectively, under varying concentration ratio of the corresponding ions. In contrast to our previous studies,[20-23] relaxation of the counterions cannot be used as a general reliable method to determine the coupling degree because the counterions in TMANa and TMAC are completely dissociated at the concentrations used (usually below 5 mmol/L) and almost so are those in C8 and A10. However, longitudinal ^{1}H NMR relaxation in the organic ions in TMANa and TMAC and in particular translation self-diffusion coefficient of the same ions measured by pulsed-gradient-field spin−echo (PGSE) were found to be quite reliable. Under conditions of fast exchange between free and bound states (cf., e.g., ref 23), the intensity of the signal in longitudinal relaxation decays exponentially

$$I_z(t) = I_z(t_0) \exp(-R_1 t) \qquad R_1 = \varphi R_{1F} + (1 - \varphi)R_{1B} \quad (17)$$

where R_{1F} and R_{1B} are the respective relaxation rates in the free
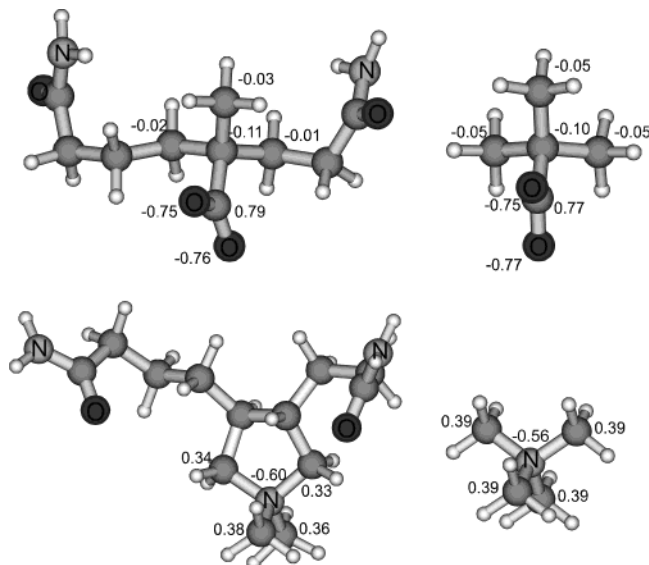
Figure 5. Mulliken charges on the decisive heavy atoms (attached hydrogen charges included) according to ab initio SCF HF/6-31G(d) calculations.

and bound states and φ is the molar fraction of the free ions. In a quite analogous way,[23] the intensity decay in PGSE is also exponential

$$I(g) = I(0) \exp(-\xi Dg^2) \qquad D = \varphi D_F + (1 - \varphi)D_B \quad (18)$$

where $\xi = \gamma^2 \delta^2 (\Delta - \delta/3)$ (γ is the gyromagnetic ratio, and δ and Δ are the lengths of the gradient pulse and the diffusion period) and φ and both subscripts have the same meaning as above. Both eqs 17 and 18 are good approximations if (i) the correlation time of exchange is substantially lower than the corresponding time window of the method and (ii) the values of R_{1F}, R_{1B}, D_F, and D_B do not change with changing molar ratio β of the reacting components. As in particular the translation diffusion coefficients D strongly depend on the macroscopic viscosity η of the system, we held the concentration of the given polyion constant at the value 5×10^{-3} mol/L (all concentrations are in molar equivalents of the charged groups) and changed the concentration of the complementary model in the range $0.5-10.0 \times 10^{-3}$ mol/L. As the viscosity slightly changed even under this arrangement, we introduced a correction for this change as shown below.

Both intensity dependences, i.e., the time dependence in the longitudinal relaxation and the squared field gradient dependences, were strictly monoexponential in all cases as shown in some examples in Figures 6 and 7. Hence the exchange between free and bound states is fast enough to ensure that eqs 17 and 18 are good approximations. We thus can write

$$\varphi = \frac{X - X_B}{\xi X_F - X_B} \quad (19)$$

where X stands for R_1 or D. We use a correction $\xi = X_w/X_{wF}$, where X_w and X_{wF} stand for R_1 or D of HOD (residual protons in heavy water) in the given system and in the solution of the free reactant, respectively. In the case of R_1, the correction is insubstantial and it is permissible to use $\xi = 1.0$ (in the case of small molecules and fast rotations, the value of microscopic viscosity η_R in the Debye formula for the rotational diffusion coefficient $D_R = 9kT/2\pi r^3 \eta_R$ clearly is different from the macroscopic viscosity η in the Stokes−Einstein formula $D = kT/6\pi r\eta$).

Electrostatic Coupling of Complementary Polyions

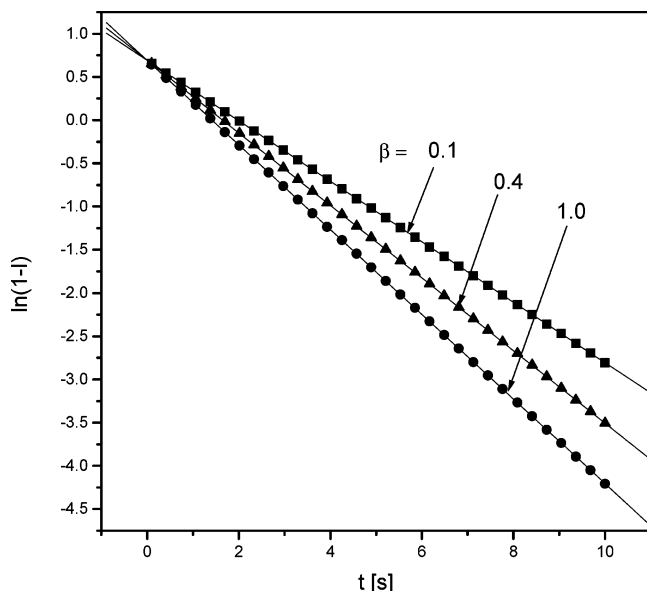*J. Phys. Chem. B, Vol. 107, No. 44, 2003* **12171**

Figure 6. Semilogarithmic time dependence of signal intensity in longitudinal relaxation of the ^{1}H NMR signal of TMANa in its mixtures with PDADMAC in D$_2$O at 300 K ([C]$_0$ = 5 × 10^{-3} mol/L, $\beta \equiv$ [a]$_0$/[C]$_0$ = 0.1, 0.4, 1.0).
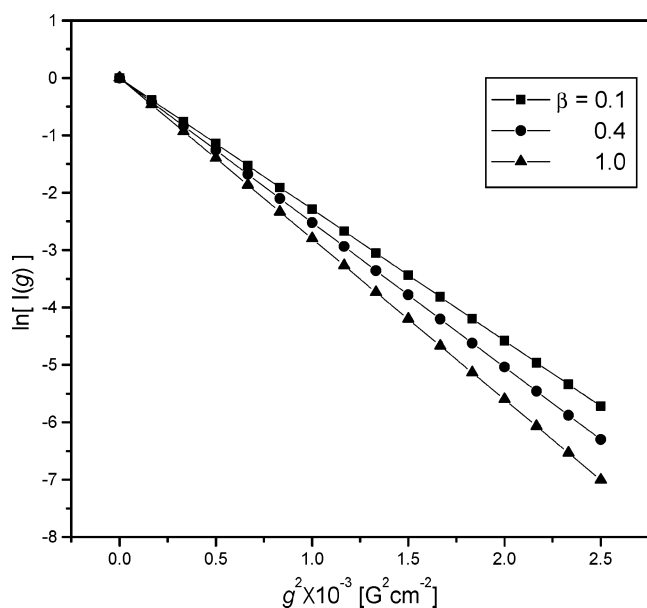

Figure 8. Values of K_2 obtained for coupling of TMANa with PDADMAC and C8. Full squares and circles obtained from PGSE; hollow ones obtained from relaxation ([C]$_0$ = 5.0 × 10^{-3} mol/L, β = [a]$_0$/[C]$_0$; 300 K in D$_2$O).
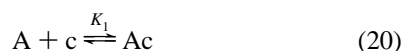

Figure 7. Squared gradient dependence of the logarithm of signal intensity in ^{1}H NMR PGSE of TMAC in its mixtures with PMANa in D$_2$O at 300 K (δ = 1.0 ms, Δ = 150 ms, [A]$_0$ = 5 × 10^{-3} mol/L, $\beta \equiv$ [c]$_0$/[A]$_0$ = 0.1, 0.4, 1.0).

Figure 9. Values of K_1 obtained for coupling of TMAC with PMANa and A10. Full squares and circles obtained from PGSE; hollow ones obtained from relaxation ([A]$_0$ = 5.0 × 10^{-3} mol/L, β = [c]$_0$/[A]$_0$; 300 K in D$_2$O).

of cooperativity in binding. Considering this, we can write

$$K_1 = \frac{1 - \varphi}{\varphi\{[A]_0 - (1 - \varphi)[c]_0\}} \qquad (22)$$

and

$$K_2 = \frac{1 - \varphi}{\varphi\{[C]_0 - (1 - \varphi)[a]_0\}} \qquad (23)$$

where [x]$_0$ means the initial concentration of the species x. Figure 8 shows the results of equilibrium coupling of PDADMAC or C8 coupled with TMANa; Figure 9 shows those for PMANa or A10 with TMAC.

These results can be summarized in three points: (i) the equilibrium constants K_1 and K_2 are generally quite low; despite

Consider now the formal equilibria

$$A + c \underset{}{\overset{K_1}{\rightleftharpoons}} Ac \qquad (20)$$

and

$$C + a \underset{}{\overset{K_2}{\rightleftharpoons}} Ca \qquad (21)$$

where A and C are anionic and cationic groups of the polymers, a and c are anionic or cationic low-molecular-weight models, and Ac and Ca are the coupling products, respectively. We call these equilibria formal because eqs 20 and 21 ignore the role of counterions and the known influence of ionic strength. They do not describe the system fully but they can be used as criteria
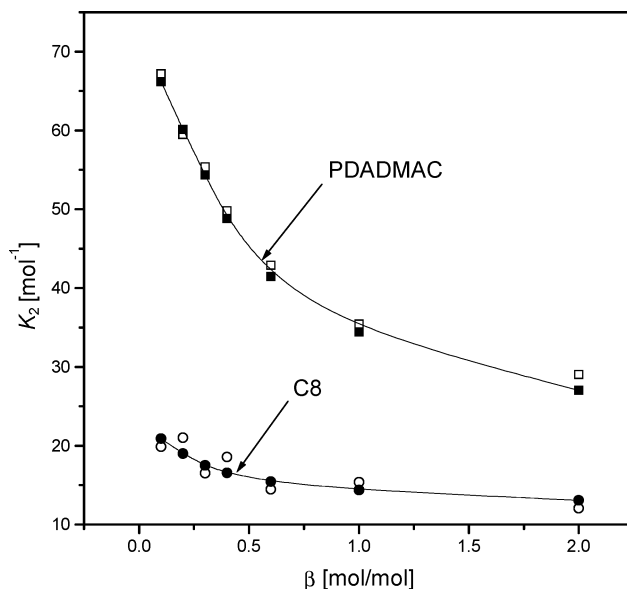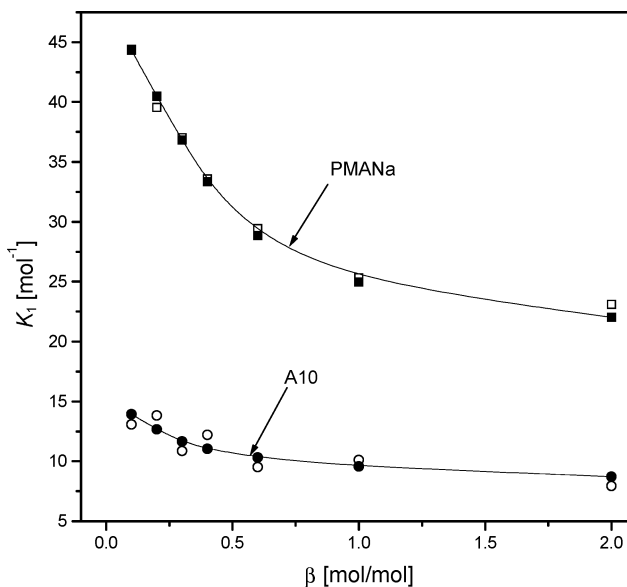
their apparently high numerical value, they correspond to coupling degrees α in the range $0.01-0.2$. (ii) Their values are generally lower for the weakly charged polyions (C8 or A10) than for the strongly charged analogues (PDADMAC or PMANa); they are also generally lower for TMAC than for TMANa. (iii) These "constants" are not constant but generally decrease with increasing β ($\beta \equiv [a]_0/[C]_0$ or $\beta \equiv [c]_0/[A]_0$).

From these points, (i) is not surprising as only a meager entropy gain can be achieved by substituting our low-molecular models for the original counterions. Nevertheless, this small gain is evidently larger for the densely charged polyions PDADMAC and PMANa than for those with dilute charge (C8 and A10), which explains point (ii). On margin, it should be noted that coupling of, e.g., DADMAC groups with original Cl$^-$ counterions appears to be energetically more favorable than that with TMA$^-$ ions. According to our MNDO calculations on a DADMA$^+$ heptamer coupled in vacuo with either Cl$^-$ or TMA$^-$ ions, the value of coupling enthalpy for one DADMA$^+$ unit is ΔH is -188.32 and -178.18 kcal/mol; i.e., the coupling is 10.14 kcal/mol in favor of Cl$^-$ ions. Hydration, which could not be adequately described in this type of calculation, could shift these values appreciably. Nonetheless, these values indicate that there is no special affinity of TMA$^-$ ions to couple with the DADMA$^+$ groups.

Point (iii) can have several different reasons, but the main one is indicated by the relatively lower decrease of the "constants" K with increasing β for both weakly charged polyions C8 and A10. The increase in β means higher ionic strength of the system, i.e., lower tendency to counterion condensation. This generally lower tendency to bind counterions is also expressed in a lower coupling degree with the relatively small ions of TMANa or TMAC.

From the point of view of the main theme of this paper, point (iii) is of primary importance: the decrease of both K_1 and K_2 with increasing β shows a lack of cooperativity in the binding of both models to the respective polyions. We can conclude that, in the absence of secondary effects such as hydrophobic interaction,[23] the binding of lone ionic groups is rather contraoperative or, at the very least, without any noticeable cooperativity.

Binding of the Complementary Weakly Charged Polyions. Assuming that TMANa is a good model of a charged unit of A10 copolymer (rather than TMAC for the charged unit of C8), we should expect the equilibrium constant K of the coupling of independent complementary groups of A10 and C8 to be the same as K_2. In the previous section, we obtained some scatter of its values, but in the range of β from 0.4 to 2.0, where its measurement is more precise, the average value is $K_2 = 15.1 \pm 0.15$ mol^{-1} at 300 K. We thus use the value $K = 15.1$ mol^{-1} in the following simulations. Defining the coupling degree $\alpha = [CA]/[A]_0$ and the ratio $\beta = [A]_0/[C]_0$ for the case of $[A]_0 \leq [C]_0$, we easily find for a simple equilibrium of mutually independent groups C + A $\leftrightarrow$ CA with the equilibrium K

$$\alpha = \frac{\psi - \sqrt{\psi^2 - 2\zeta K[C]_0}}{\zeta} \tag{24}$$

with $\psi = K[C]_0(1 + \beta) + 1$ and $\zeta = 2\beta K[C]_0$. Figure 10 shows illustrative simulations of α for different β at various values of $[C]_0$. For $\beta = 1.0$, the expected coupling degree α of the groups is 0.065 and 0.015 for 5.0 and 1.0×10^{-3} mol/L. The average number of ionic groups per one macromolecule is 597 for C8 and 376 for A10, respectively. Thus about 39 and 9 ionic groups of an average C8 macromolecule should form an electrostatic
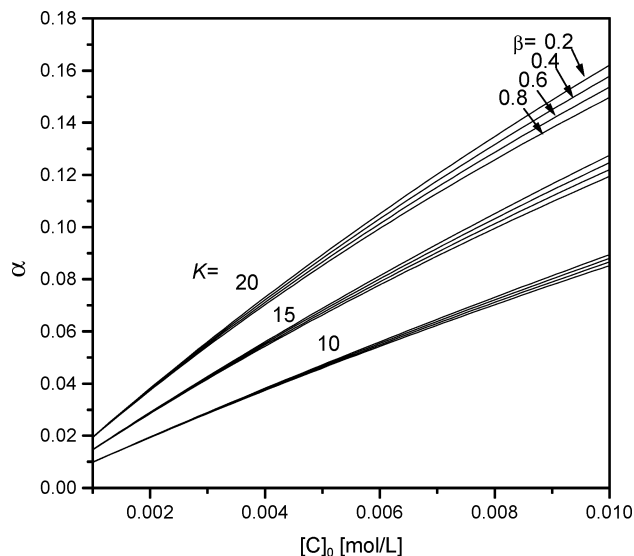

Figure 10. Simulated values of $\alpha = [CA]/[A]_0$ for the equilibrium C + A $\leftrightarrow$ CA at indicated values of equilibrium constant K and $\beta = [A]_0/[C]_0$ (for $K = 15$ and 10 the values of β and orders of curves are analogous).

bond at 5.0 and 1.0×10^{-3} mol/L, respectively. In both cases, a very weak but existent physical linking of several macromolecules is expected (each link being separated on average by 190 and 828 neutral or ionic groups, respectively), presumably leading to water-swollen colloid particles. In reality, even very slow mixing (1 mL of A10 solution added to 1 mL of C8 solution at the rate of 0.2 mL/h under vigorous stirring) of the components at $\beta \geq 0.8$ leads to a cloudy "solution" with a small amount of sediment at $[C]_0 = 5 \times 10^{-3}$ mol/L and to crosslinked particles, which flocculate and eventually sediment at $[C]_0 = 1 \times 10^{-3}$ mol/L. Therefore, we studied the polyion coupling in the range of β from 0.1 to 0.6.

The determination of the coupling degree α is somewhat more difficult in this case as the PFG diffusion methods cannot be effectively used here. Thus only ^{23}Na and ^{35}Cl NMR relaxation are left. At the first glance, the former method is less advantageous than the latter as $R_{1B} - R_{1F}$ is only 2.08 s^{-1} for ^{23}Na but 173.0 s^{-1} for ^{35}Cl. In reality, the former is more reliable due to much lower sensitivity of the ^{35}Cl resonance and the consequent interference of noise even when accumulating very high numbers (typically 4×10^4) of scans. However, both methods agree to within 8% of the actual value of α, which is good enough to demonstrate the effects pursued in this paper.

Figure 11 shows the actually measured values of α at various values of β in the range $0.1-0.6$ and $[C]_0 = 5 \times 10^{-3}$ and 1×10^{-3} mol/L. The experimental values differ drastically from those predicted from analogy with low-molecular-weight models but are even generally *higher* for *lower* $[C]_0$. Both features are in blatant contrast with the model based on independent ionic groups. As shown in Figure 11, our experimental data are much better described by the model of proximity-mediated cooperativeness expressed by eqs 8 and 9, using $d = 0.25$ nm and $m_A + m_C = 21.5$. As one can expect, our simple model describes experimental data in a very approximate way only. In reality, there are surely more complicated constraints not only of the moving apart but also of the encounter of groups A and C. This in particular should be the case at higher concentration (Figure 9), where the probability of binding of more than two macromolecules (in a *scrambled egg* complex) is evidently higher. In such a case, some of the groups could be prevented from coupling on purely geometrical reasons.

TABLE 1: ^{14}N Chemical Shifts δ_N and Half-Widths $\Delta\nu_{1/2}$, and ^{13}C Chemical Shifts δ_C and $T_{1\rho}$ Values of the α-Protons in Acrylamide Units of the Parent C8 and Coupling Products with A10 Immediately after Mixing and after 4 months (2 mmol/L in D$_2$O, 300 K)

	up to 3 days				after 4 months			
β	δ_N, ppm	$\Delta\nu_{1/2}$, Hz	δ_C, ppm	$T_{1\rho}$, s	δ_N, ppm	$\Delta\nu_{1/2}$, Hz	δ_C, ppm	$T_{1\rho}$, s
0.0	−267.9	6.8	171.54	0.197	−268.0	6.9	171.55	0.198
0.2	−268.1	6.9	171.56	0.196	−267.3	16.2	170.48	0.174
0.4	−268.0	6.9	171.61	0.196	−267.4	16.4	170.23	0.169
0.6	−268.1	7.0	171.58	0.198	−267.4	17.2		0.158
0.8	−268.1	6.9	171.61	0.197	−267.5	17.8		0.152
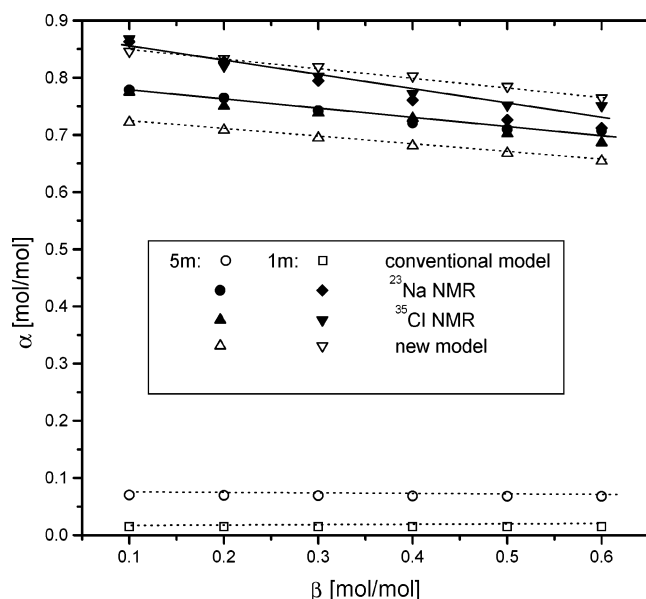
Figure 11. Simulated (for $K = 15.1$ mol^{-1}) and experimentally established values of α for equilibrium C + A $\leftrightarrow$ CA at various values of β at $[C]_0 = 5 \times 10^{-3}$ (5 *m*) and 1×10^{-3} (1 *m*) mol/L (D$_2$O, 300 K).

Notwithstanding such imperfections, the prediction of the model is right in order of magnitude, in contrast to the conventional model based on free ionic groups.

Search for Other Possible Sources of Cooperative Behavior. As already said in the Introduction, no usual interactions, which were recognized as additional driving forces of cooperativity, can be expected in our case. The phase effects of shifting the equilibrium simply by flocculation and sedimentation of the product can be excluded by the fact that the products are soluble, in particular at the concentration of 2 mmol/L ionic groups. Similarly, there is no reason to expect hydrophobic interaction as the major part of the interacting polymers C8 and A10 consists of hydrophilic acrylamide units.

However, interactions of acrylamide units of complementary chains via hydrogen bonding cannot be a priori excluded, although we have not observed any interaction between poly-(acrylamide) chains at such low concentrations in water. The methods of NMR appropriate for detecting such interactions surely are (i) ^{15}N and ^{14}N shift, (ii) ^{13}C carbonyl shift, (iii) broadening of the ^{14}N signal due to perturbed symmetry and lower mobility, and (iv) increased transverse relaxation rate of the α-proton in the acrylamide unit due to decreased mobility. From these, ^{15}N is quite excluded due to sensitivity and ^{13}C gives poor spectra even after 48 h of accumulation. As for the α-proton mobility, the conventional spin−echo method using the CPMG sequence meets some problems due to scalar coupling to β-protons but the $T_{1\rho}$ experiment is viable. We used on-resonance pulsed spin−lock with mean field $\omega_1 = 2.5 \times 10^3$ rad/s. Table 1 shows the values of ^{14}N chemical shifts δ_N

and signal half-widths $\Delta\nu_{1/2}$, ^{13}C=O chemical shifts δ_C, and α-H $T_{1\rho}$ values measured within 3 days after mixing and again after about 4 months (the latter having been provoked by the fact that some samples at higher concentration (10 mmol/L) clouded or even sedimented after several weeks). All these results refer to 2 mmol/L ionic groups in D$_2$O at 300 K.

As clearly seen from the table, there are no signs of H-bonding or any other interaction of acrylamide units immediately after mixing, when the coupling degree α was determined. On the contrary, there seems to be some interaction (possibly combined with rearrangement of the product) after a long time. The nature of this interaction is not quite clear, but it almost certainly involves H-bonding.

From the point of view of the present paper, the only relevant results are those measured at approximately the same time as α was determined. We can conclude that we have no evidence of additional nonionic interactions which could significantly cooperate with the ionic ones and thus explain the effects described in this paper.

Conclusions

We have shown here that cooperative electrostatic binding between weakly charged complementary polyions takes place. The effect is observed even in the cases where charge dilution by interleaving neutral chains removes characteristic counterion condensation from the original polyions, suppressing thus the entropic boost gained by counterion liberation. We have shown that neither of the polyions examined here, namely poly(sodium methacrylate)-*co*-(acrylamide) containing 10 mol % ionic groups and poly(*N*-diallyldimethylammonium chloride)-*co*-(acrylamide) containing 8 mol % ionic groups, exhibits perceptible counterion condensation in dilute aqueous solution. As expected, binding of low-molecular-weight models of the respective complementary ionic groups, i.e., tetramethylammonium chloride and sodium pivalate, to these polyions exhibits low equilibrium and no cooperativity. However, binding of the corresponding polyions is cooperative and leads to surprisingly high coupling degrees in dilute solutions.

We proposed a simplified theoretical model that can explain this behavior. The model shows that the binding equilibrium can be substantially shifted due to restraints of motion exerted on the coupling groups by the nearby already coupled groups. This effect is described here for a one-to-one molecular complex of the polyions, but the model could be generalized to complexes containing a larger number of macromolecules. The quantitative predictions of the model fit the experimental data in a semi-quantitative way only, but we believe that they could be improved if a detailed description of motional constraints was included in the model.

We believe that the model named *insular kinetics* in the Introduction and outlined in this paper in a simplified way can be applied to a wide variety of interacting macromolecular systems. However, intensive studies of systems that could be

better defined and thus described in detail are needed before it can be established as a verified theory.

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