Biomacromolecules **2006,** *7,* *281−287*

Specific Interactions versus Counterion Condensation. 1. Nongelling Ions/Polyuronate Systems

Ivan Donati,* Attilio Cesàro, and Sergio Paoletti

Department of Biochemistry, Biophysics and Macromolecular Chemistry, University of Trieste, Via Licio Giorgieri 1, I-34127 Trieste, Italy

Received September 5, 2005; Revised Manuscript Received October 28, 2005

The characteristics of the interaction between nongelling divalent cations (typically Mg^{2+}) and polyuronates have been explored by means of isothermal calorimetry. In particular, three polyuronates mimicking separately guluronan (polyguluronate, polyG), mannuronan (polymannuronate, polyM), and polyalternating (polyMG), the three block-components of natural alginate samples, have been treated with divalent ions, and the enthalpy of mixing was determined for different values of the $[M^{2+}]/[\mathrm{Polym}]_{\mathrm{rep.unit}}$ ratio. Despite the absence of a site-specific chemical bonding between the two, as confirmed by circular dichroism spectroscopy, a substantial deviation of the experimental enthalpy of mixing from the theoretical behavior, as predicted by the classical counterion condensation (CC) theory, was observed. Such deviation has been interpreted in terms of a “generic” nonbonding affinity of the condensed divalent counterion for the polyelectrolytes. The mathematical formalism of the CC theory was extended to include a contribution to the (reduced) free energy and enthalpy arising from the counterion affinity, $g^{\mathrm{aff},0}$ and $h^{\mathrm{aff},0}$, and allowed the parametrical calculation of the fraction of divalent counterions condensed as function of the reduced thermodynamic quantity $g^{\mathrm{aff},0}$. A best fit procedure of the experimental enthalpy of mixing allowed the $g^{\mathrm{aff},0}$ and $h^{\mathrm{aff},0}$ pair to be estimated for each of the different polyuronates considered, revealing differences in the three samples. In qualitative terms, the results obtained seem to suggest a notable contribution of the desolvation process (i.e., release of structured water as a consequence of the interaction between the divalent counterion and the uronate group) to the enthalpy of affinity for polyM which is counterbalanced and overcome by an ion pairing term (i.e., partial formation of ion−ion and/or ion−dipole bonds) for polyG and polyMG, respectively.

Introduction

Ionic polysaccharides exhibit interesting physicochemical properties which render them of the utmost interest for the food and the biomedical industry. Among them, the ability to form stable wall-to-wall hydrogels in the presence of millimolar amounts of specific divalent cations addresses polyuronates, such as alginate and pectate, as suitable materials for applications in the field of microencapsulation of living organisms.[1,2] Alginate is a collective term for a family of linear copolymers of 1→4 linked β-D-mannuronic acid (M) and α-L-guluronic (G) acid arranged in a block wise pattern along the chain with homopolymeric regions of M (“M blocks”) and G (“G blocks”) residues interspersed with regions of alternating structure (“MG blocks”). The ionotropic gelation of alginate has been described, based on X-ray fiber diffraction data from dehydrated specimens, using the so-called “egg-box” model.[3,4] According to the latter, junction zones are formed between 2/1 helical chains of G sequences that present cavities suitable to accommodate specific divalent cations. Recently, it has been reported that calcium binding properties cannot be exclusively allocated to G blocks, as alternating sequences do share similar behavior upon treatment with calcium ions.[5]

Despite the remarkable amount of work performed and the knowledge achieved on the interaction between alginates and gel forming (or “binding”) divalent counterions (in particular calcium and barium), little attention has been addressed to the description and implications of the interaction with the “nonbinding” ions (such as magnesium). However, it is well-known that long range electrostatic interactions of small ions in solution with highly charged linear polyelectrolytes affect their structure, stability, and solubility. In fact, electrostatic interactions can account for conformational changes, modification of the degree of association of counterions, and/or interchain interactions.[6−10]

Among the theoretical approaches most commonly used to describe the counterion distribution around polyelectrolytes, i.e., nonlinear Poisson−Boltzmann equation, Monte Carlo simulations, and the counterion condensation (CC) theory, the latter can be regarded as a simple and handy tool to describe the physicochemical behavior of a wide variety of polyelectrolyte−counterion pairs.[11,12] Moreover, it proved to be flexible enough to be modified to encompass more sophisticated aspects such as chain flexibility[13] and the description of systems of mixed counterions with different valence.[14] Recently, a mathematical formalism has been developed, within the framework of the CC theory, to account for specific affinity of counterions toward polyelectrolytes. It predicted a shifting of the equilibrium distribution between free and condensed counterions, thus paralleling the counterion selectivity detected in several experimental results.[6,15,16]

The present work is aimed at assessing the nonbonding affinity (“binding affinity” in the terminology introduced by Record and co-workers[17]) of divalent counterions for polyuronates by comparing theoretical predictions and experimental results. The interactions considered here are comparatively “weak”, inasmuch as they do not involve the formation of rigid

---

* Corresponding author. Tel: +39 040 558 2403. Fax: +39 040 558 3691. E-mail: donati@bbcm.units.it.

10.1021/bm050646p CCC: $33.50

complex structures, which might even require the simultaneous pairing of conformationally ordered stretches of different chains (e.g., "covalent" bonding at a specific site).[18] The theoretical model has been constructed by exploiting a mathematical approach sketched in the theory section that takes into account the presence of mixed counterions of different valence, namely z_i and z_j, and of a contribution to the total Gibbs energy arising form a nonbonding affinity of the z_j-valent counterion. Both theoretically and experimentally, we focused on the effect of nonjunction forming z_j-valent counterions on polyuronates. In particular, the availability of polymers mimicking G, MG, and M blocks, namely "PolyME6" (or polyG), polyalternating, (or polyMG), and mannuronan (or polyM), respectively, makes it possible to separately highlight the features of nonbonding interactions of divalent cations with the three different components of natural alginate samples. The suitability of magnesium ions for the three polyuronates selected, and calcium ions for mannuronan, as nonjunctions forming counterions has been assessed by means of circular dichroism.

This group has already dealt with the problem of introducing a specific affinity term into the frame of the CC theory.[6,16,19] Initially, the interest was focused only onto the Gibbs free energy term, g^{aff}, to account for the preferred selectivity for calcium with respect to sodium of poly (methacrylic acid) and dextran sulfate.[19] The separation of g^{aff} into its enthalpic and entropic components was investigated for potassium with κ-carrageenan in DMSO[16] and for cesium with the same polymer in water.[6] However, this was achieved by analyzing the dependence of the transition melting temperature on the (logarithm of the) ionic strength using only one type of counterion, rather than studying the thermal evolution on mixing a "reference" polyelectrolyte salt with increasing amounts of the second "specific" salt. The isothermal calorimetry approach will be considered here because it offers some advantages, as it was already repeatedly maintained by this group.[7,10,20] Among others, one should recall high sensitivity, comparatively little use of sample, and demonstrated insensitivity to gel formation, which dramatically affects, e.g., optical techniques. Calorimetric data, if correctly interpreted within the framework of a proper theory, may be able to simultaneously provide information both on the free energy and on the enthalpy changes involved in counterion/ polyion interactions.

In the present work, the comparison between the theoretical and experimental enthalpy of mixing with divalent counterions allowed both the intrinsic free energy and enthalpy of affinity toward the divalent counterions to be estimated, revealing differences of behavior among the polyuronates considered. In our strategy, the assessment of the nonbonding affinity contribution in the condensation of divalent cations on polyuronates represents the starting point from which the more challenging further description of the ion-induced chain pairing process (i.e. junction formation) can be tackled.

Materials and Methods

The preparation and main compositional features of mannuronan, polyalternating, and polyME6 have been described elsewhere.[5] Calcium perchlorate, magnesium perchlorate, and sodium perchlorate were from Aldrich Chemical Co. (Milwaukee, WI).

Circular Dichroism Spectroscopy. Circular dichroic spectra of the sodium forms of the polymers mannuronan, polyalternating, polyME6, and polygalacturonic acid were recorded in 0.05 M $NaClO_4$ (polymer concentration of 3.5×10^{-3} monomol/L, pH $\sim$ 7) with a JASCO J-700 spectropolarimeter. A quartz cell of 1-cm optical path length was used, and the following setup was used throughout: bandwidth, 1 nm; time constant, 2s; scan rate, 20 nm/min. Four spectra, corrected for the background, were averaged for each sample. The spectrum of each sample was recorded prior and after the addition of $Mg(ClO_4)_2$ at different $[M^{2+}]/[Polym]_{\mathrm{rep.unit}}$ (R_j) molar ratio. In the case of mannuronan, also $Ca(ClO_4)_2$ was used.

Calorimetry. Calorimetric experiments were performed, for mannuronan, polyalternating, and polyME6, using a LKB 10700−2 batch type microcalorimeter. The variation of the measured property (ΔH^{mix}) was determined upon mixing a polymer solution with a solution containing the divalent cation to a specific $[M^{2+}]/[Polym]_{\mathrm{rep.unit}}$ (R_j) ratio. All of the experiments were performed with a final polymer concentration equal to 3.5×10^{-3} monomol/L and in the presence of 0.05 M $NaClO_4$. The results have been corrected taking into account the enthalpy of dilution of both the polymers and the salts.

Theory

As usual within the framework of the counterion condensation theory,[21] the polyuronates are modeled as an infinite line with charges separated by a distance b_{str} that corresponds to the average charge distance as projected onto the polymer axis. The characteristic parameter of the CC theory is the linear charge density ξ given by (eq 1)

$$\xi = \frac{l_{\mathrm{B}}}{b_{\mathrm{str}}} \tag{1}$$

where $l_{\mathrm{B}} = (e^2/(\epsilon k_{\mathrm{B}} T))$ is the Bjerrum length, e is the electronic charge, ϵ is the dielectric constant of the bulk solvent, k_{B} is the Boltzmann constant, and T is the absolute temperature.

One of the basic features of the CC theory concerns the existence of a critical value of the charge density of the polyelectrolyte, ξ_{crit}, above which a fraction r of counterions of valence z_i is condensed onto the polyelectrolyte chain. It follows that a fraction (rz_i) of the fixed charge on the polyion (q_{struct}, assumed to be unitary) will be effectively shielded. Thus, for the "effective" charge on each ionized site on the polyelectrolyte, in the presence of only one type of counterions, it holds

$$q_{\mathrm{eff}} = q_{\mathrm{struct}}(1 - rz_i) \tag{2}$$

However, when mixed counterion polyelectrolyte solutions are considered, both species of counterions are found in the condensation volume.[14] Therefore, the total condensed fraction r can be written as

$$r = r_i + r_j = r(x_i + x_j) \quad \text{and} \quad x_j = 1 - x_i \tag{3}$$

where r_i and r_j stand for the fractions of condensed counterions of valence z_i and z_j, respectively.

In this case, it holds

$$q_{\mathrm{eff}} = q_{\mathrm{struct}}(1 - r_i z_i - r_j z_j) \tag{4}$$

In the polyelectrolyte solution in the presence of mixed counterions of different valence, the (molar) fraction of condensed counterions, r, and the fraction of either type of counterion, $r_{i,j}$, are obtained from the condition of stability of the system at infinite dilution (i.e., avoiding divergence at polymer concentration approaching to zero; eq 5)

$$r = \frac{1}{(z_i x_i + z_j(1 - x_i))}\left[1 - \frac{1}{\xi(z_i x_i + z_j(1 - x_i))}\right] \tag{5}$$

For a detailed description of the limiting condition treatment and the above-reported equation see ref 14.

Following the so-called “chemical model” as first developed by G. S. Manning, the total (reduced) molar excess free energy of purely polyelectrolytic nature, g^{ion}, is factored into a purely electrostatic term, g^{el}, and a reduced molar free energy of mixing of mobile species, g^{mix}. In general, g^{x} is a particular (“x”) Gibbs free energy normalized per mole of polymer fixed charge and reduced by RT, where R is the universal gas constant

$$g^{\mathrm{ion}} = g^{\mathrm{el}} + g^{\mathrm{mix}} \qquad (6)$$

In general, the final form of g^{el} will be

$$g^{\mathrm{el}} = -\xi {q_{\mathrm{eff}}}^{2} \ln(1 - e^{-Kb}) \qquad (7)$$

which, in the case of a solution with mixed counterions of valences z_i and z_j, reads

$$g^{\mathrm{el}} = -\xi(1 - r(z_j - x_i(z_j - z_i)))^2 \ln(1 - e^{-Kb}) \qquad (8)$$

where K is the reciprocal of the Debye length.

The entropy of mixing contribution of the different mobile ionic species (counterions of valence z_i and z_j that are either free to move in the bulk solution or condensed onto the polyelectrolyte chain, similions and solvent molecules) to the change in Gibbs energy, g^{mix}, is given by

$$g^{\mathrm{mix}} = g_i^{\mathrm{cond}} + g_j^{\mathrm{cond}} + g_i^{\mathrm{free}} + g_j^{\mathrm{free}} + g_{\mathrm{simil}} + g_{\mathrm{solv}} \qquad (9)$$

The different entropic contributions can be written as[14]

$$g_i^{\mathrm{cond}} = rx_i \ln\left(\frac{rx_i}{(1 + R_i)V_{\mathrm{p}}C_{\mathrm{p}}}\right)$$

$$g_j^{\mathrm{cond}} = r(1 - x_i) \ln\left(\frac{r(1 - x_i)}{R_j V_{\mathrm{p}} C_{\mathrm{p}}}\right)$$

$$g_i^{\mathrm{free}} = (1 + R_i - rx_i) \ln\left(\frac{(1 + R_i - rx_i)}{(1 + R_i)(1 - V_{\mathrm{p}}C_{\mathrm{p}})}\right)$$

$$g_j^{\mathrm{free}} = (R_j - r(1 - x_i)) \ln\left(\frac{(R_j - r(1 - x_i))}{R_j(1 - V_{\mathrm{p}}C_{\mathrm{p}})}\right)$$

$$g_{\mathrm{simil}} = (R_i + z_j R_j) \ln\left(\frac{1}{(1 - V_{\mathrm{p}}C_{\mathrm{p}})}\right)$$

$$g_{\mathrm{solv}} = r$$

where R_i and R_j stand for the molar ratios between the concentrations of the ith (e.g., uni-univalent) and jth (e.g., bi-univalent) salt, respectively, and that of the polymer, C_{p}; V_{p} is the molar volume of the condensed phase, expressed in liters per mole of fixed charge.

In the addition to the above-reported equations, a contribution to the reduced free energy accounting for the affinity, g_j^{aff}, of the uronic acids toward j-type condensed cations (with respect to i-type) has to be taken into account, eq 10

$$g_j^{\mathrm{aff}}(R_j) = r(R_j)(1 - x_i(R_j))g^{\mathrm{aff},0} \qquad (10)$$

where $g^{\mathrm{aff},0}$ is the intrinsic reduced free energy of affinity (per mole of condensed counterion) of the j-valent counterion toward the polyelectrolyte, assumed to be independent of the state of occupation of neighboring “sites”. It should be underlined that eq 10 implies that the affinity is *not* associated with any long lasting, geometrically well-defined “bonding”; thus, the j-type condensed counterions are in an exchange equilibrium with the “free” noncondensed counterions of the same type in solution, i.e., outside V_{p}, under the boundary condition that their fraction ($r_j = rx_j$) is constant for constant physicochemical conditions. Any (tolerated) reduction of their diffusion coefficient as a consequence of affinity for the polymer structure would anyway keep them free to move within the condensation volume without reaching permanent immobilization on a well-defined site on the polymer.

The combination of eq 5 with the conditions of free energy minimization for the system with two different counterions of mixed valence, i.e., $\partial g^{\mathrm{ion}}/\partial r = 0$ and $\partial g^{\mathrm{ion}}/\partial x_i = 0$, leads to a system of three equations in three unknowns (r, x_i, and V_{p}) which can be parametrically determined at equilibrium as a function of the intrinsic (reduced) free energy of affinity, $g^{\mathrm{aff},0}$.

The analytical expression of g^{ion} allows determining the total reduced enthalpy of purely electrostatic origin, h^{ion}, expressed (per mole of polyelectrolyte charged group) as

$$h^{\mathrm{ion}} = \frac{\partial(g^{\mathrm{ion}}/T)}{\partial(1/T)}$$

Considering that the term g^{mix} corresponds to the contribution of the different mobile species to the entropy of mixing, h^{ion} reduces to

$$h^{\mathrm{ion}}(R_j) = h^{\mathrm{el}}(R_j) + h^{\mathrm{aff}}(R_j) = h^{\mathrm{el}}(R_j) + r_j(R_j)h^{\mathrm{aff},0} \qquad (11)$$

with

$$h^{\mathrm{el}} = \frac{\partial(g^{\mathrm{el}}/T)}{\partial(1/T)} = -\frac{1}{2}\,\xi(1 - r_i x_i - r_j x_j)^2\left(1 + \frac{\mathrm{d}\ln D}{\mathrm{d}\ln T}\right)\left[2\ln(1 - e^{-Kb}) + \frac{Kb}{e^{Kb} - 1}\right] \qquad (12)$$

and $h^{\mathrm{aff},0}$ as the intrinsic (reduced) enthalpy of affinity (per mole of condensed counterion). In the absence of any contrary evidence and for sake of simplicity, $h^{\mathrm{aff},0}$ will be supposed to be a constant, independent of R_j, i.e. of the state of occupation of neighboring “sites”. Although the theoretical treatment has been accomplished using i- and j-type of counterions, with no loss of generality, we will focus, in the results and discussion section, on the case $z_i = 1$ and $z_j = 2$.

Results and Discussion

The mathematical description of the affinity-driven interaction between the polyelectrolyte and the counterions has been accomplished extending the original polyelectrolyte model; this approach has already been exploited and demonstrated its usefulness and reliability in different cases.[16,19] As an example, the thermodynamic parameters for the conformational transition have been successfully calculated in the case of the specific Cs^+-induced ordering and chain-pairing of κ-carrageenan.[6]

Along this line, the present paper is aimed at assessing the occurrence and implications of affinity-driven interaction between polyuronates and nonbonding divalent cations. To this purpose, three polyuronate samples, namely mannuronan, poly-alternating and polyME6, have been considered in view of their resemblance with the three different sequences found in natural alginate samples. In fact, mannuronan, obtained from an epimerase-negative mutant of *Pseudomonas fluorescens*, is composed exclusively of M residues and thus strictly mimics

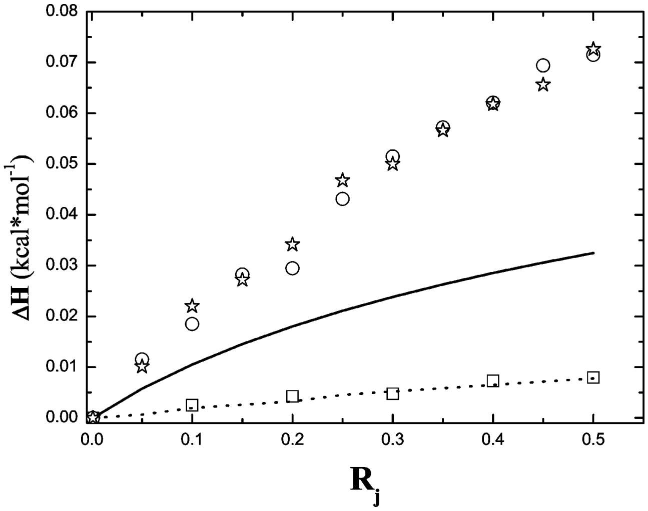

Figure 1. Experimental enthalpy of mixing of mannuronan with (○) Mg^{2+}, (☆) Ca^{2+}, and (□) Na$^+$ In the latter case, a sodium concentration equaling the ionic strength of the solution at each R_j was used. (- – -) Theoretical enthalpy of mixing for Na$^+$ treated mannuronan calculated according to ref 20. (—) Theoretical enthalpy of mixing for Ca^{2+} treated mannuronan calculated by using $g^{aff,0}$ and $h^{aff,0} = 0$. In both cases, the polymer concentration was 3.5×10^{-3} monomol/L and the monovalent supporting salt was 0.05 M.

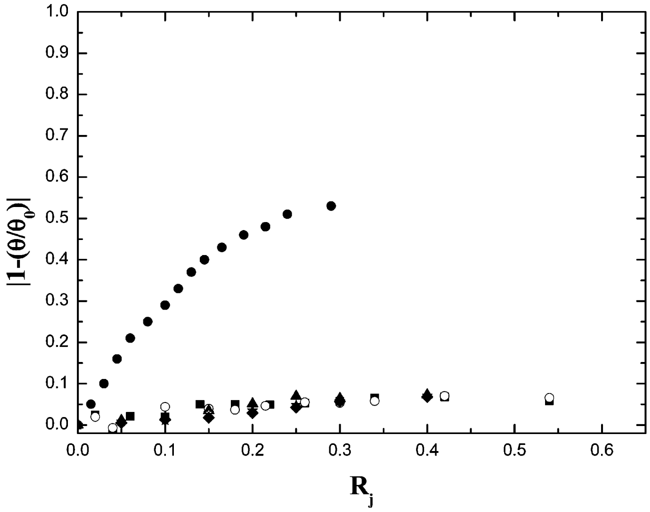

Figure 2. Dependence on R_j of the specific change in ellipticity, measured at 210 nm, of (■) mannuronan, (▲) polyME6 (i.e., guluronan, polyG), (♦) polyalternating, and (☆) pectate treated with magnesium ions and of (○) mannuronan treated with calcium ions. For comparison, the specific change in ellipticity (●) for calcium treated pectate (site-specific "covalent" bonding) is reported.

the M blocks. On the other hand, polyalternating and polyME6, achieved by an enzymatic strategy, closely resemble the MG and G blocks, respectively (see ref 5 for details). Since the scope of this work is to study affinity, but *nonbonding*, interactions, magnesium ions have been selected as divalent counterions for all the polyuronates considered, while calcium ions were considered exclusively in the case of mannuronan, for which Ca^{2+} ions are known *not* to give rise to strong interactions nor to gel formation. Sodium ions have been used as nonspecific monovalent counterions, whose interactions with the polymer are assumed to be (and will be shown so) only electrostatic.

Figure 1 reports the enthalpy variation (data points) measured upon mixing mannuronan with Mg^{2+}, Ca^{2+}, and Na$^+$ ions and shows a nonsurprising different behavior of the polyelectrolyte–ion system depending on the valence of the counterion. However, it is interesting to compare the results with the theoretical enthalpy change upon mixing mannuronan with monovalent (ref 20, dotted line) and divalent counterions (full line), the latter calculated neglecting all kind of affinity interactions. Although there is a very good agreement between the theoretical prediction and the experimental data in the former case, i.e., with monovalent ions, a substantial discrepancy arises when comparing the system containing divalent counterions. It was resorted to CD spectroscopy to verify if such extra enthalpy contribution could stem from a strong, site-specific, bonding of the divalent counterion by the polyuronates. Only negligibly small changes in specific ellipticity of the polyuronate solutions were noticed (Figure 2) upon addition of calcium to mannuronan, at variance with the large CD variations that had been reported for the strong interactions of calcium with alginates and pectate.[3,5,22] (For comparison purposes, the ellipticity curve for Ca^{2+} with pectate was experimentally reproduced in this work and the results are reported in Figure 2 as well). Similar nonspecific effects were observed for mixing magnesium with mannuronan, polyME6, and polyalternating. In addition, Figure 2 unveils the modest variation of specific ellipticity upon adding magnesium to pectate; the CD data then confirm the well-known similarity of behavior of pectate with alginate-related polyuronates, namely their polyguluronate component.[23,24] The numerical values of the ordinate of Figure 2 are so similar as to preclude any reasonable attempt at differentiating among the various cases. The whole of these chiro-optical results unambiguously points to the *absence* of a site-specific chemical bonding of magnesium with all alginate-related polymers and with pectate (and of calcium with mannuronan). The observed deviations of the enthalpy of mixing from the theoretical behavior (see Figure 1) must then stem from a contribution arising from a "generic" nonbonding affinity of the condensed divalent counterions for the investigated uronate polyelectrolytes.

The model presented in the theory section allows for the straightforward calculation of the contribution of each species to the total fraction of condensed counterions (r_i and r_j for the mono- and the divalent counterions, respectively) and of the thermodynamic functions of interest (i.e., g^{ion} and h^{ion}). The calculation of the latter appears very appealing since a direct comparison with experimental results (ΔH^{mix}) is possible.

However, it should be recognized that in the model developed

$$h^{ion} = h^{ion}(r_{i,j}, h^{aff,0}), \qquad h^{el} = h^{el}(r_{i,j})$$

and

$$r_{i,j} = r_{i,j}(g^{aff,0})$$

It follows that the value of $g^{aff,0}$ strongly affects the fraction of j-valent (divalent in our case) condensed counterions (Figure 3) and, hence, the numerical value of both h^{el} and h^{ion}. In particular, negative values of $g^{aff,0}$ will favor the relative binding of divalent counterions over the monovalent ones, producing values of r_j larger than those predicted on the basis of the theoretical model without any affinity. As a final consequence, the entropic term to the Gibbs free energy of affinity can be computed

$$s^{aff,0} = g^{aff,0} - h^{aff,0} \Rightarrow \overline{\Delta S}^{aff,0} = s^{aff,0}R$$

To find the best set of $g^{aff,0}$ and $h^{aff,0}$ to fit the experimental data of the enthalpy of mixing, the calculation of r_j was performed trying with $g^{aff,0}$ values from 0 to -8 (corresponding to a range of $G^{aff,0}$ ($= g^{aff,0}RT$) between 0 and $-$ 4.7 kcal(mol divalent ion)$^{-1}$). The best fit between the experimental and the

Table 1. Structural Parameters of Polyuronates and Thermodynamic Parameters of Their Affinity for Magnesium Ions[a]

sample	b_{str}[b] (Å)	x	$g^{aff,0}$ [c]	$h^{aff,0}$ [c]	$s^{aff,0}$ [c]	$\Delta G^{aff,0}$ [d] (kcal mol^{-1})	$\Delta H^{aff,0}$ [d] (kcal mol^{-1})	$\Delta S^{aff,0}$ [d] (kcal mol^{-1})
polyME6 (guluronan)	4.44	1.61	−6	0.07	6.07	−3.55	41.4	3.59
polyalternating	4.80	1.49	−2	−0.04	1.96	−1.18	−23.7	1.16
mannuronan	5.17	1.38	−0.5	1.24	1.74	−0.30	734	1.03

[a] For mannuronan, also with calcium. Conditions: aqueous $NaClO_4$ 0.05 M, 25 °C. [b] Calculated on the basis of the composition of each polymer by using the values 4.35 Å and 5.17 Å for guluronate and mannuronate residues, respectively.[20] [c] Intrinsic reduced thermodynamic parameters of affinity calculated per mole of condensed counterion from the minimization procedure. $g^{aff,0} = h^{aff,0} - s^{aff,0}$. [d] Intrinsic total thermodynamic parameters of affinity calculated per mole of condensed counterion from the minimization procedure. $\Delta G(H)^{aff,0} = g(h)^{aff,0}RT$; $\Delta S^{aff,0} = s^{aff,0}R$. The error associated with the calculation of the intrinsic free energy of affinity term was estimated to be approximately 15%. The error associated the intrinsic entropy of affinity was found to range from 15% (polyalternating) to 8% (mannuronan)

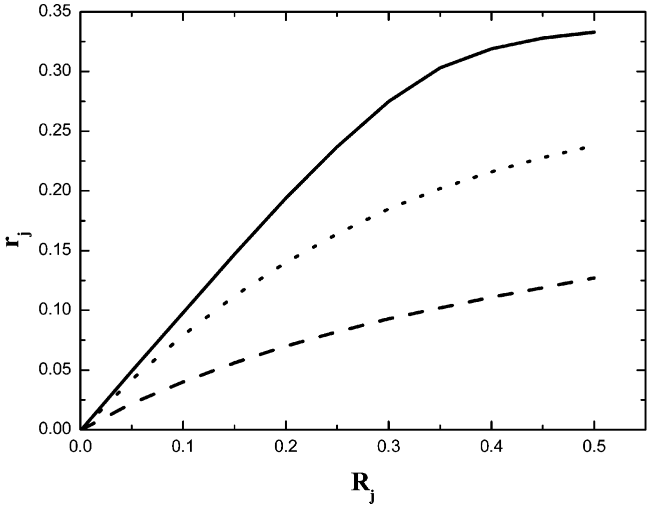

Figure 3. Dependence on R_j of the fraction of j-valent counterions condensed on the polyelectrolyte, r_j, calculated for polyME6 (i.e., guluronan, polyG) by using $g^{aff,0}$ equal to (—) −1, (- - -) −4, and (—) −6, respectively.

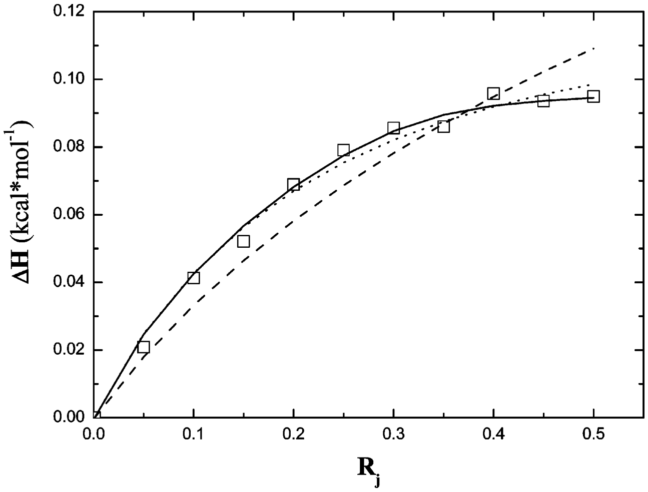

Figure 4. Best fit between the (□) experimental enthalpy of mixing of polyME6 and Mg^{2+} and theoretical curves calculated by using $g^{aff,0}$ equal to (—) −1, (- — -) −4, and (—) −6, respectively.

theoretical values of enthalpy of mixing was performed for each r_j−$g^{aff,0}$ pair and provided the value of $h^{aff,0}$ under the assumptions

$$\Delta h^{ion}(R_j) = h^{ion}_{R_j}(R_j) - h^{ion}_{R_j=0}$$

and

$$\Delta H^{mix}_{theor}(R_j) = \Delta h^{ion}(R_j)RT$$

The minimization procedure was obtained by means the Find−Minerr subroutine of the Mathcad 5.0 software program (Mathsoft Inc., Cambridge, MA)

Figure 4 reports the best fit results obtained for polyME6 by using different $g^{aff,0}$ values. It can be clearly seen that, by setting a value for the intrinsic (reduced) free energy of affinity ($g^{aff,0}$) equal to −1, the prediction of the theoretical model deviates substantially from the experimentally measured enthalpy variations. Conversely, by increasing $g^{aff,0}$ toward more negative values, i.e., $g^{aff,0} = -6$ ($\sim$−3.5 kcal (mol divalent ion)$^{-1}$), the agreement between the experimental and the theoretical data is remarkably higher pointing at the important role of the r_j−R_j dependence. The same analysis was repeated for the other polyuronates under consideration, i.e., polyalternating and mannuronan, determining, from the best fitting procedure, a unique (and different) value of $g^{aff,0}$ and, consequently, of $h^{aff,0}$ which led to the best agreement between the theoretical predictions and the experimental data. From the results reported in Table 1 it can be concluded that, albeit always within a model of "generic" nonbonding affinity, still significant differences do exist in the intrinsic free energy and enthalpy of affinity toward the j-valent counterions among the investigated polyuronates. However, it does not seem likely that such differences result from the corresponding small variations in b, the average charge distance projected on the polymer axes, and thus in the charge density parameter ξ (see Table 1).

Although proper care should be taken as to the absolute value of these intrinsic thermodynamic quantities, some conclusions can tentatively be drawn. In particular, it is interesting to note that the absolute value of the intrinsic (negative) free energy of affinity ($g^{aff,0}$) decreases along the series PolyME6 (G blocks) > polyalternating (MG blocks) > mannuronan (M blocks), likely reflecting the partial lack of ion selectivity by the latter. In fact, a very similar ΔH^{mix}−R_j dependence is recorded for mannuronan regardless of the divalent counterion used, i.e., Mg^{2+} or Ca^{2+} (see Figure 1). At variance, polyuronates which are known to display a dramatically different behavior with different divalent cations (PolyME6 and polyalternating form stable hydrogels with Ca^{2+} as a consequence of a site-specific covalent-like bonding) are characterized by a relevant value of the intrinsic free energy affinity (see Table 1). Along this line, it seems feasible to predict that pectate, a gel-forming polymer structurally resembling polyG sample ($b = 4.43$Å and $\xi = 1.61$), should share with the latter similar figures concerning the affinity toward divalent cations. This point will be the subject of future work.

An opposite tendency, with respect to $g^{aff,0}$, is detected for $h^{aff,0}$ which decreases along the series mannuronan ≫ polyME6 > polyalternating becoming surprisingly negative in the latter case.

The graphical representation of this trend is given in Figure 5, panel I. It reports the dependence of ΔH^{mix}_{theor}, ΔH^{el}, and

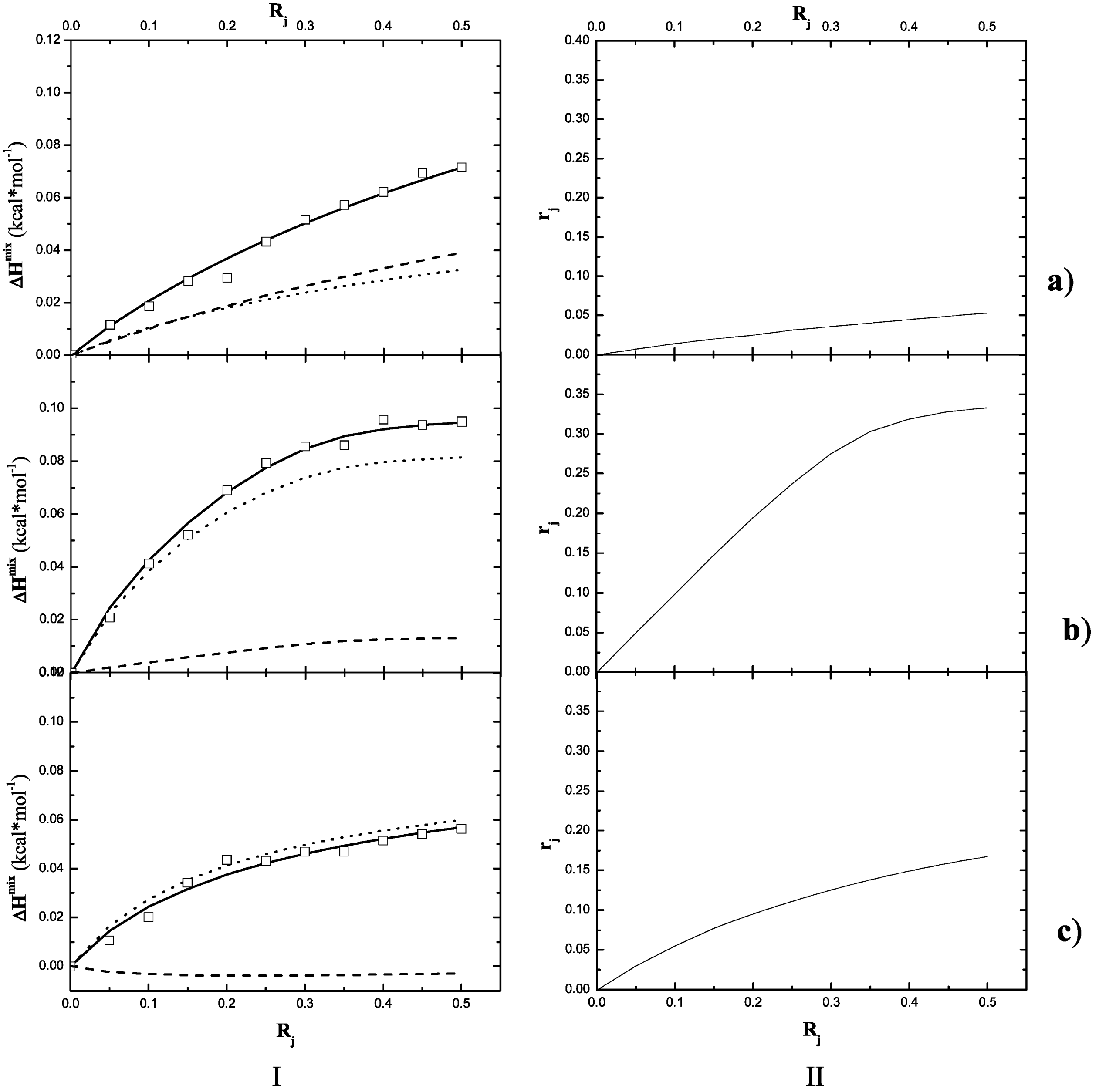

Figure 5. Dependence on R_j of (panel I) (—) $\Delta H^{\text{Mix}}_{\text{theor}}$, (- - -) ΔH^{el}, and (— —) ΔH^{aff} and (panel II) fraction of *j*-valent (divalent) ions condensed on the polyelectrolyte, r_j, calculated for (a) mannuronan, (b) polyME6, and (c) polyalternating using the values of intrinsic free energy and enthalpy of affinity reported in Table 1. Experimental values (□) of enthalpy of mixing, ΔH^{Mix}, of the polyuronates with Mg^{2+} ions are also reported.

ΔH^{aff} (which is directly proportional to $h^{\text{aff},0}$) on R_j, calculated from the values of the intrinsic thermodynamic quantities of Table 1. It can be noted that for both mannuronan and polyME6 the electrostatic component as well as the affinity one are always endothermic. At variance, a negative contribution from the intrinsic enthalpy of affinity is needed to correlate theoretical predictions and experimental results for polyalternating. In addition, Figure 5, panel II, reports the r_j−R_j dependence obtained for the three polyuronates. It is immediately apparent that the fitted dependence of r_j on R_j closely parallels the ΔH^{mix}−R_j relation, pointing to the importance of the fraction of divalent cations condensed on the polyelectrolyte in the determination of the overall thermodynamic properties of the solutions.

To attempt a qualitative explanation of the present result, it might be revealing considering that the thermodynamic quantity $g^{\text{aff},0}$ can be reasonably supposed to be made up by two contributions arising from (i) desolvation (release of structured water from the hydration shells of both the counterion and the uronic moiety), g^{desolv} and (ii) ion pairing, g^{ip}, respectively (see eq 13)

$$g^{\text{aff},0} = g^{\text{desolv}} + g^{\text{ip}} \tag{13a}$$

Similarly

$$h^{\text{aff},0} = h^{\text{desolv}} + h^{\text{ip}} \tag{13b}$$

and

$$s^{\text{aff},0} = s^{\text{desolv}} + s^{\text{ip}} \tag{13c}$$

The former process is expected to be accompanied by a rather large and positive enthalpic value (h^{desolv}) because of the rupture of several ion−dipole (water) bonds, which would be paralleled, at the same time, by a large and positive value of s^{desolv} of “cratic” origin, i.e., due to the acquired translational freedom

Table 2. Schematic Representation of the Sign and Magnitude of the Contributions of Desolvation, h^{desolv}, and of Ion Pairing, h^{ip}, to the (Reduced) Intrinsic Enthalpy of Affinity, $h^{\mathrm{aff},0}$

sample	h^{desolv}	h^{ip}	$h^{\mathrm{aff},0}$
polyME6	+++	- -	+
polyalternating	+++	- - -	-
mannuronan	+++	-	++

of the released water molecules. On the other side, h^{ip}, i.e., the enthalpic contribution stemming from the counterion "confinement", is expected to be negative due to the partial formation of ion−ion bonds (between the divalent ion and the uronate groups) and/or ion−dipole bonds (between the divalent ion and the lone pairs of oxygen atoms on the polymer). Correspondingly, s^{ip} is expected to be negative, although definitely smaller than s^{desolv} in absolute value, because of the partial immobilization of the ionic partners in the, albeit flickering, confinement. According to the values reported in Table 1, the enthalpy of desolvation would largely dominate when mannuronan is considered, while the ion-pairing enthalpic contribution seems to (slightly) dominate in the case of polyalternating. A balance of these two terms appears to happen in the case of polyME6. A schematic and qualitative representation of the different contributions in the affinity driven condensation of magnesium ions on polyuronates is reported in Table 2.

Before concluding, it seems appropriate to restate that all thermodynamic functions of affinity are relative to the interaction of the monovalent counterions (here: sodium ions) with the polyelectrolyte. The free energy of affinity of the latter ions, $(g^{\mathrm{aff},0})_{\mathrm{Na}}$, is conventionally taken as equal to zero, as well as $(h^{\mathrm{aff},0})_{\mathrm{Na}}$ (and hence $(s^{\mathrm{aff},0})_{\mathrm{Na}}$). It corresponds to assume that the affinity interactions of Na^+ ions with the polyion are that of counterions as point-charges in the original CC treatment, being their reference state that of the equivalent 1:1 electrolyte in the Debye−Hückel limiting conditions. The good matching between the theoretical predictions and the experimental results of ΔH^{mix} for NaCl (see Figure 1) proves that these assumptions are sound.

Conclusions

It has been shown that a neat and self-consistent modification of the CC theory of linear polyelectrolytes is able to account for the presence of effects that have conventionally attributed to "specific" interactions between counterions and polyions. This is despite some established ideas of the past, when sometimes it was even stated that the very presence of "specific interactions" would make it impossible to tackle counterion/polyion systems by means of modern polyelectrolyte theories.

The case of condensed divalent counterions showing a preferential "affinity" for polyanions with respect to monovalent ones has been considered, with no loss of generality. Results from isothermal microcalorimetry experiments have been nicely reproduced using reasonable values of the fundamental thermodynamic functions of the affinity event.

The investigated systems are those of the "weak" interaction of magnesium ion with three ionic polysaccharides representing compositional limiting cases of alginates. The study has shown that chemical composition and monomer sequence produce different values of the enthalpic and of the entropic component of the affinity interaction, then bearing accumulation of divalent ions around the polymer to a different extent (i.e., different $\Delta G^{\mathrm{aff},0}$ values). However, such known differences appear just as a "fine-tuning" of the more general process of counterion condensation. The result is not trivial at all. Rather, it contributes with an important step forward to the challenging task of the thermodynamic description of the interactions of gel-promoting counterions (namely calcium) with polyuronates (in particular, pectate and the alginates). Additional terms will necessarily be introduced into the theory to account for the much stronger interactions shown by those systems as macroscopically manifested by the very phenomenon of gelation, albeit preserving the present "background" of nonbonding specific interactions. Work is in progress along this line; both the development of a mathematical description of the association process and a detailed comparison with experimental results for gelling polyuronates will be addressed in forthcoming papers.

Acknowledgment. The authors thank Prof. Gudmund Skjåk-Bræk of the Institute of Biotechnology, University of Trondheim (NTNU), for providing the mannuronan, polyalternating, and polyME6 samples.

References and Notes

(1) Lim, F.; Sun, A. M. *Science* **1980**, *210*, 908.
(2) Soon-Shiong, P.; Feldman, E.; Nelson, R.; Komtebedde, J.; Smidsrød, O.; Skjåk-Bræk, G.; Espevik, T.; Heintz, R.; Lee, M. *Transplantation* **1992**, *54*, 769.
(3) Morris, E. R.; Rees, D. A.; Thom, D.; Boyd, J. *Carbohydr. Res.* **1978**, *66*, 145.
(4) Grant, G. T.; Morris, E. R.; Rees, D. A.; Smith, P. J. C.; Thom, D. *FEBS Lett.* **1973**, *32*, 195.
(5) Donati, I.; Holtan, S.; Mørch, Y. A.; Borgogna, M.; Dentini, M.; Skjåk-Bræk, G. *Biomacromolecules* **2005**, *6*, 1031.
(6) Paoletti, S.; Gamini, A.; Vetere, A.; Benegas, J. C. *Macromol. Symp.* **2002**, *186*, 141.
(7) Cesàro, A.; Delben, F.; Paoletti, S. *J. Chem. Soc., Faraday Trans. 1* **1988**, *84*, 2573.
(8) Ray, J.; Manning, G. S. *Langmuir* **1994**, *10*, 2450.
(9) Ray, J.; Manning, G. S. *Macromolecules* **1997**, *30*, 2450.
(10) Paoletti, S.; Delben, F.; Cesàro, A.; Grasdalen, H. *Macromolecules* **1985**, *18*, 1834.
(11) Manning, G. S. *J. Phys. Chem.* **1984**, *88*, 6654.
(12) Paoletti, S.; Smidsrød, O.; Grasdalen, H. *Biopolymers* **1984**, *23*, 1771.
(13) Paoletti, S.; Cesàro, A.; Guidugli, S.; Benegas, J. C. *Biophys. Chem.* **1991**, *39*, 9.
(14) Paoletti, S.; Benegas, J. C.; Cesàro, A.; Manzini, G.; Guidugli, F.; Crescenzi, V. *Biophys. Chem.* **1991**, *41*, 73.
(15) Benegas, J. C.; Paoletti, S.; Cesàro, A.; van den Hoop, M. A. G. T.; van Leeuwen, H. P. *Biophys Chem.* **1992**, *42*, 297.
(16) Paoletti, S.; Benegas, J. C.; Pantano, S.; Vetere, A. *Biopolymers* **1999**, *50*, 705.
(17) Bleam, M. L.; Anderson, C. F.; Record, M. T., Jr. *Proc. Natl. Acad. Sci. U.S.A.* **1980**, *77*, 3085.
(18) Porasso, R. D.; Benegas, J. C.; van den Hoop, M. A. G. T.; Paoletti, S. *Phys. Chem. Chem. Phys.* **2001**, *3*, 1057.
(19) Benegas, J. C.; Paoletti, S.; van den Hoop, M. A. G. T. *Macromol. Theory Simul.* **1999**, *8*, 61.
(20) Paoletti, S.; Cesàro, A.; Delben, F.; Crescenzi, V.; Rizzo, R. in *Microdomains in Polymer Solutions*; Dubin, P., Ed.; Plenum Press: New York, 1985; p 159.
(21) Manning, G. S. *Q. Rev. Biophys.* **1978**, *11*, 179.
(22) Kohn, R.; Sticzay, T. *Czech. Chem. Commun.* **1977**, *42*, 2372.
(23) Rees, D. A., Welsh, E. J., *Angew. Chem., Int. Ed. Engl.* **1977**, *16*, 214.
(24) Ravanat, G.; Rinaudo, M. *Biopolymers* **1980**, *19*, 2209.

BM050646P