^{23}Na n.m.r. studies of partially neutralized poly(galacturonic acid)

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The dependence of sodium ion binding to poly(galacturonic acid) on degree of neutralization has been determined by ^{23}Na n.m.r. and calculated using the Poisson–Boltzmann theory for the system without excess salt over the polyion concentration range 5–50 mM (monomer basis). The relative fraction of bound sodium ions depends on the polyion concentration and the degree of neutralization. The agreement between the experimental and calculated fraction bound is excellent for the lowest concentration at degree of neutralization greater than about 0.2 and for even the highest concentration at degree of neutralization greater than about 0.6.

(Keywords: sodium binding; poly(galacturonic acid); degree of neutralization; ^{23}Na n.m.r.)

INTRODUCTION

The amount of counterion territorially bound or 'atmospherically condensed' to a polyion in solution has been formulated by Manning[1] in terms of the dimensionless charge density parameter, ξ, defined as:

$$\xi = e^2/(4\pi kTl\varepsilon_0\varepsilon_r) \qquad (1)$$

where e is the elementary charge, l is the projection of the intercharge distance on the axis of the polyion assumed to be a cylinder, k is the Boltzmann constant, T is the temperature (K), ε_0 is the permittivity in vacuum and ε_r is the dielectric constant of the solvent. This charge density parameter is the ratio of the Bjerrum length, B, to l and the theory predicts binding only where $B/l > 1$. Thus, according to Manning's theory, the number of counterions territorially bound on the polyion per unit charge is given by:

$$P_b = 1 - (1/z\xi) \qquad (2)$$

where z is the valence (without sign) of the counterion.

Although a body of experimental evidence[2] supports the validity of equation (2) in concentrated solutions and in the presence of salts, it appears to be inadequate at low concentrations and in the absence of excess salts. This was demonstrated by studies of the ^{23}Na n.m.r. line shapes of aqueous solutions of sodium poly(galacturonate), poly(mannuronate) and poly(guluronate) by Grasdalen and Kvam[3]. These authors extracted the correlation times, τ_c, from spectral line shapes which become distinctly non-Lorentzian for τ_c exceeding 0.5 ns. Using the concentration dependence of τ_c, they showed that the data were consistent with the Poisson–Boltzmann (PB) theory[4] and a theory developed by Halle *et al.*[5] for the quadrupolar relaxation of the counterions, as testified by the relative constancy of the ^{23}Na quadrupolar coupling constant at different polyelectrolyte concentrations calculated from the mathematical formulation of this theory. Subsequently, ^{23}Na n.m.r. studies[6,7] of competitive binding of Mg^{2+} and Na^+ to poly(galacturonate) in mixed counterion systems at different polyelectrolyte concentrations and in the absence of excess salts revealed trends in competitive binding which were compatible with the PB rather than with Manning's theory.

Another way to examine compatibility with these theories experimentally, using certain assumptions, is to monitor the line width of ^{23}Na n.m.r. spectra as a function of the degree of neutralization, α, of a polyacid with sodium hydroxide.

In Manning's theory, P_b depends only on the parameter l for any given solvent and temperature. As the weak polyacid is neutralized by the strong base, the projection of the interchange distance on the axis of the cylinder indicated by l^α, increases as α decreases. Assuming uniform distribution of charges of the fully extended chain, it is given by:

$$l^\alpha = l^*/\alpha \qquad (3)$$

where l^* is the projection of the distance between adjacent acid groups on the axis of the cylinder and the dimensionless parameter of equation (1) is related to α by:

$$\xi^\alpha = e^2\alpha/(4\pi kTl^*\varepsilon_0\varepsilon_r) \qquad (4)$$

and P_b in equation (2) becomes P_b^α. In the case of NaOH as the neutralizing base, $z = 1$ and:

$$P_b^\alpha = 1 - (1/\xi^\alpha) \qquad (5)$$

Thus in Manning's theory, P_b^α depends on α but is independent of concentration.

In the PB theory P_b^α depends not only on l^α for a given solvent and temperature but also on the total concentration of charged groups on the polyion in the solution. Let this concentration be denoted by c_m^α. Then if the concentration of the neutralizable weak acid groups on the polyacid is denoted by c_m^*, c_m^α is given by:

$$c_m^\alpha = c_m^*\alpha \qquad (6)$$

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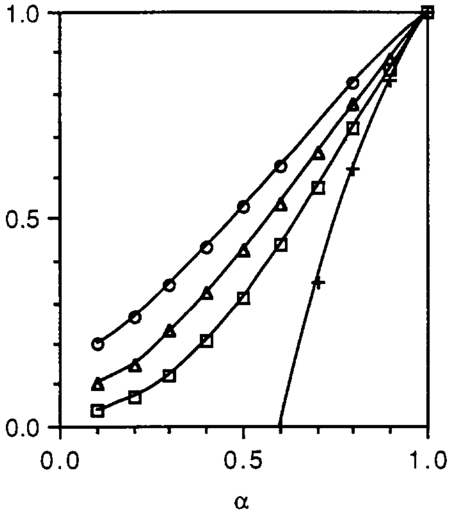

Figure 1 Calculated Γ^α *versus* α for PGA using the Poisson–Boltzmann (PB) and Manning theories: ○, PB theory, $c_m^* = 50$ mM; △, PB theory, $c_m^* = 20$ mM; □, PB theory, $c_m^* = 5$ mM; +, Manning theory

The PB theory introduces the radius of the cylinder representing the polyion, a, and the radius of a cylindrical cell in the solution, R, whose dimensions are chosen so as to give c_m^* (refs 3 and 5). For any given polyacid, R becomes:

$$R = [(\pi l^* c_m^* N)1000]^{-1/2} \qquad (7)$$

where N is the Avogadro number. Since

$$l^* c_m^* = l^\alpha c_m^\alpha \qquad (8)$$

R is constant for all α in a given titration. However, for a given polyacid, R depends on c_m^*, which introduces a dependence of P_b^α on c_m^* in the PB theory that does not occur in Manning's theory.

In the absence of excess salts P_b in equation (2) is given by:

$$P_b = X_B \qquad (9)$$

where X_B is the fraction of counterions of only one kind territorially bound to the polyion. Letting X_B^α denote the fraction of the Na$^+$ ions territorially bound to a weak polyacid for any given α and X_B^1 denote the same fraction in a fully neutralized polyacid ($\alpha = 1$), Γ^α is defined as:

$$\Gamma^\alpha = X_B^\alpha / X_B^1 \qquad (10)$$

and represents the amount of Na$^+$ bound to the polyacid neutralized to a degree α relative to the amount bound to the fully neutralized polyacid. Γ^α can be calculated by either of the two theories for any selected set of parameters.

Using the same parameters as tabulated by Moss *et al.*[8] for sodium poly(galacturonate) at 300.2 K, we have calculated Γ^α for several concentrations of poly(galacturonic acid) (PGA). These results are plotted in *Figure 1* over the range of α from 0.1 to 1, using the PB and Manning's theory. The difference between the two theories is clearly demonstrated by the curves. Whereas Γ^α is independent of c_m^* and zero below $\alpha = 0.604$, i.e. the region of $\xi < 1$, calculated by Manning's theory, it is dependent on c_m^* and non-zero even at the lowest concentration ($c_m^* = 5$ mM) down to at least $\alpha = 0.1$ calculated by the PB theory.

Γ^α can be experimentally determined by measuring the ^{23}Na n.m.r. line widths at half-height using the following relation based on a two-site model[3,9]:

$$\Delta\nu_{1/2} = (1 - X_B)\Delta\nu_{1/2,F} + X_B\Delta\nu_{1/2,B} \qquad (11)$$

where $\Delta\nu_{1/2,F}$ and $\Delta\nu_{1/2,B}$ are the line widths at half-height which characterize the free and bound states at any α, respectively, and $\Delta\nu_{1/2}$ is the experimentally observed line width.

Let $\Delta\nu_{1/2}^\alpha$ be the line width measured for any given c_m^* at the degree of neutralization α and let $\Delta\nu_{1/2}^1$ be the corresponding line width for the same c_m^* at complete neutralization ($\alpha = 1$). Then:

$$\Delta\nu_{1/2}^\alpha = (1 - X_B^\alpha)\Delta\nu_{1/2,F} + X_B^\alpha\Delta\nu_{1/2,B} \qquad (12)$$

and

$$\Delta\nu_{1/2}^1 = (1 - X_B^1)\Delta\nu_{1/2,F} + X_B^1\Delta\nu_{1/2,B} \qquad (13)$$

assuming that $\Delta\nu_{1/2,B}$ has the same value in equations (12) and (13), i.e. that the relaxation time, τ_c, and quadrupole coupling constant of ^{23}Na are the same for all values of α, equations (10), (12) and (13) lead to an experimentally determined Γ^α, Γ_{exp}^α:

$$\Gamma_{exp}^\alpha = [\Delta\nu_{1/2}^\alpha - \Delta\nu_{1/2,F}]/[\Delta\nu_{1/2}^1 - \Delta\nu_{1/2,F}] \qquad (14)$$

Experimentally, $\Delta\nu_{1/2,F}$ is determined from a solution of NaCl at the same temperature and with the same solvent.

Grasdalen and Kvam[3] reported transverse ^{23}Na n.m.r. relaxation rate measurements for PGA at different degrees of neutralization (see Figure 7 in ref. 3) but at two relatively high concentrations, without analysing the data with reference to theory. They ascribed the apparently anomalous plot at the higher concentration to some possible processes affecting either or both the τ_c and a change in the nature or the amount of counterion binding.

The work described in this paper is focused on the ^{23}Na n.m.r. transverse relaxation measurements at three concentrations of poly(galacturonate) ($c_m^* = 5$, 20 and 50 mM) with the object of comparing experimental results with theoretical predictions.

EXPERIMENTAL

PGA ($M_W = 30\,000$) was obtained from Polysciences, Inc. To prepare solutions of different α, a portion of a solution of PGA was titrated with NaOH to obtain a solution of its sodium salt, i.e. NaPGA, with $\alpha = 1$. After dilution of both solutions to the desired concentration, PGA and NaPGA were mixed in appropriate proportions to obtain a solution with the desired α and concentration. Care was taken to avoid sodium contamination by preparing solutions in plastic bottles and using quartz n.m.r. tubes. A fresh solution was prepared for each experiment and stored overnight before obtaining the n.m.r. spectrum.

The ^{23}Na n.m.r. spectra were recorded with a Bruker ACP-200 spectrometer at 52.939 MHz using optimized pulse angle and repetition time. The delay between pulses was at least 10 times greater than the transverse relaxation time, T_2. Temperature was controlled within ± 0.5°C. The spectra were determined by the accumulation of pulse responses, the number ranging from 2000 for the 50 mM solutions to 3000 for the 5 mM solutions. The half-widths of the lines at half-height, $\Delta\nu_{1/2}^\alpha$, for $\alpha = 1.0$ were 66.7, 78.7 and 82.2 Hz for the 5, 20 and 50 mM PGA solutions, respectively. The line width for free

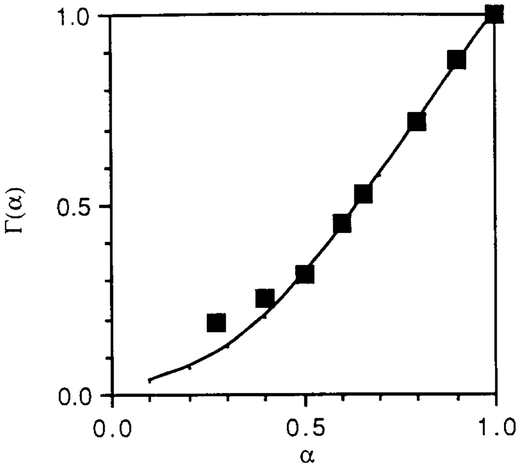

Figure 2 Γ^α *versus* α for $c_m^* = 5$ mM PGA; experimental points with curve calculated by PB theory

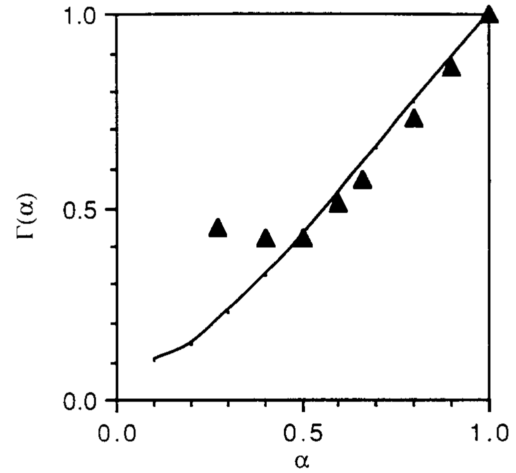

Figure 3 Γ^α *versus* α for $c_m^* = 20$ mM PGA; experimental points with curve calculated by PB theory

sodium ion, $\Delta\nu_{1/2,F}$, was 5.8 Hz, estimated using a 0.05 M solution of NaCl. Every complete set of measurements for a particular polyelectrolyte, i.e. all the solutions of different degrees of neutralization of a particular concentration, was determined under identical instrumental settings. A spectrum of a 0.05 M NaCl solution was obtained before and after the experiments with the PGA solutions to ensure that there was no change in conditions during the run. The uncertainty of the measured Γ^α_{exp} is about ± 0.04 at 5 mM PGA and about ± 0.02 at 50 mM PGA.

RESULTS AND DISCUSSION

Graphs of the Γ^α *versus* α for $c_m^* = 5$, 20 and 50 mM calculated by the PB theory and determined from the ^{23}Na n.m.r. spectra of PGA are shown in *Figures 2* to *4*. The theoretical curve provides excellent agreement with the experimental points for $\alpha > 0.3$ for the 5 mM solution, and the deviation at $\alpha < 0.3$ is small. The agreement between the theoretical and experimental Γ^α values is also excellent for the more concentrated solutions at α greater than about 0.5, i.e. for $\alpha > 0.5$ for the 20 mM solution and $\alpha > 0.6$ for the 50 mM solution. However, the deviations at α less than these critical values become much larger as the concentration increases. Although the dependence of Γ^α on c_m^* predicted by the PB theory was obtained for the experimental Γ^α at α greater than the critical values, all three experimental curves differed significantly from the theoretical curve calculated by Manning's theory in these ranges of α.

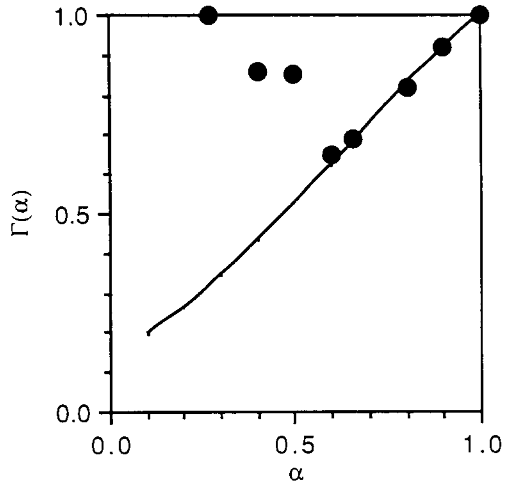

Figure 4 Γ^α *versus* α for $c_m^* = 50$ mM PGA; experimental points with curve calculated by PB theory

The deviation of the experimental Γ^α from that calculated by the PB theory below the critical value of α can possibly be attributed to a conformational change in the PGA at low pH and high concentration. A change in conformation can affect either the value of τ_c, or the magnitude of the quadrupole coupling constant, or both. The exceptionally high values of τ_c at small α obtained for poly(methacrylic acid) have been attributed to conformational change[10]. The line shapes of ^{23}Na n.m.r. obtained in this work carried out with a 200 MHz instrument are so close to Lorentzian that τ_c could not be evaluated with meaningful accuracy by the line shape method[3]. Although further investigation using a higher field instrument might permit higher accuracy in determining τ_c, the quadrupole coupling constant cannot be independently determined by any known experimental procedure.

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