$^{23}Na^+$ NMR IN SOLUTIONS OF MUCOPOLYSACCHARIDES

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1. Introduction

Polyelectrolytes have a wide occurrence in biological systems and their function is greatly influenced by abundant small ions, for example, Na^+, K^+, Mg^{2+} and Ca^{2+}. The quadrupole relaxation method for studying ion binding to macromolecules, the principles of which are in [1], should constitute a very general experimental approach to the problem of ion binding in biological systems. While the method has won wide-spread use in the field of protein chemistry [1–3], ion binding to biological polyanions like nucleic acids [4,5], humic acids [6–9] and others [10] has not been penetrated to the same depth. One reason for this is that in the case of proteins, it is much easier to isolate relaxation effects (e.g., using competition experiments) directly related to the biological function while this is not so for the polyanions. To be of any significant value, quadrupole relaxation studies of ion binding to polyelectrolytes must include studies of both longitudinal (T_1) and transverse (T_2) relaxation times as a function of the degree of ionization. Even so the analysis is not without difficulties because several factors may influence the measured relaxation rates. However, a recent analysis [11] of rather extensive $^{23}Na^+$ relaxation data for a synthetic polyanion, polymethacrylic acid (PMA), gave consistent results and provides a suitable basis for the discussion of polyelectrolyte systems in general.

Mucopolysaccharides, occurring in the extracellular matrix of connective tissues, form a group of biological polyanions for which the interactions with small cations is of great significance [12,13]; e.g., there is certainly a critical interplay between ion binding phenomena and functionally important conformational changes. The present report presents preliminary $^{23}Na^+$ NMR studies of mucopolysaccharide systems and in one case (dermatan sulfate) a closer analysis of the relaxation times is performed along the lines in [11].

2. Experimental

The $^{23}Na^+$ line-widths and chemical shifts were obtained using a modified Varian XL-100 spectrometer operating in the Fourier transform mode at 26.46 MHz. For the determination of the longitudinal (T_1) and transverse (T_2) relaxation times, a Bruker BK 322-s spectrometer operating at 23.81 MHz was used as in [11]. Experimental temperature was $29 \pm 2°C$.

Chondroitin-4-sulfate, dermatan-4-sulfate and hyaluronic acid were prepared and purified as in [14]. All inorganic chemicals used were of 'pro analysis' quality and the water was quartz distilled. Solutions were prepared by dissolving the sodium salts of the polyions to 0.2 molal concentration (in monosaccharide units) in 0.1 molal aqueous sodium hydroxide solution. To reduce the viscosity of the hyaluronate solution, it was diluted 4-fold with distilled water.

3. Results and discussion

$^{23}Na^+$FT NMR spectra were obtained as a function of the amount of added hydrochloric acid for alkaline solutions of dermatan sulfate, chondroitin sulfate and hyaluronic acid using the Varian XL-100 spectrom-

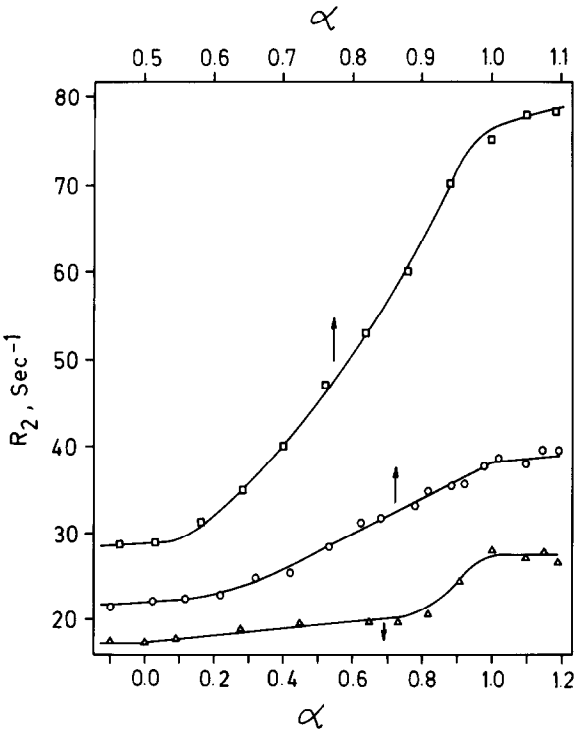

Fig.1. $^{23}Na^+$ relaxation rates, R_2 (from line-widths, in s^{-1}), as a function of the 'stoichiometric' degree of neutralization, α, for aqueous solutions of dermatan-4-sulfate (□), chondroitin-4-sulfate (○) and hyaluronate (△). The concentrations are (in monomeric units) 0.2, 0.2 and 0.050 molal, respectively. The sodium ion concentrations are for the first two substances 0.3 molal and for the hyaluronate 0.075 molal.

eter. The results are plotted as the transverse relaxation rate (R_2) as a function of the 'stoichiometric' degree of ionization of the polyanion in fig.1. (R_2 was obtained from the half height line-width, $\Delta\nu_{1/2}$, according to $R_2 = \pi\,\Delta\nu_{1/2}$; this is a convenient measure of the relaxation but as discussed below relaxation is in fact non-exponential and given by two superimposed exponentials.) These results clearly demonstrate the feasibility of the method to establish changes in counterion binding occurring as a result of both changed degree of ionization and changed chemical structure of the mucopolysaccharide. It may be recalled that both chondroitin sulfate and dermatan sulfate have one ionizable group on each monosaccharide unit and that these are alternatively sulfate and carboxylate groups; the positions of the groups differ in the two cases. Hyaluronic acid, which has much longer chains than the others, has a carboxylate group on every second monosaccharide unit and no sulfate group.

Results, like those given in fig.1, certainly contain important information on the relation between counterion binding and conformation but a detailed interpretation is not straight-forward since relaxation depends on the following three factors which cannot be separated using this type of information only:

(i) The distribution of counterions over the possible binding environments in the solution. In the simple case of a single type of binding site on the polyion, only a distinction between free and bound counterions is needed and then R_2 contains information on the fraction of ions bound, p_B.

(ii) The electric field gradients sensed by the bound counterions. This is expressed by a quadrupole coupling constant $\chi = e^2qQ/h$.

(iii) The time dependence of the field gradients. This is generally expressed by a correlation time, but for the cases considered here the averaging of the field gradients is not a simple process, and it may be useful to consider relaxation as effected by one rapid local motion and one slow motion over the dimensions of the aggregates. It is then important to consider the effect of the local motion on the effective quadrupole coupling constant.

Detailed expressions of the relaxation rates are given elsewhere [1,11,15] and here it should only be stressed that for a spin-3/2 nucleus like $^{23}Na^+$, relaxation is non-exponential for the long correlation time case. However, it was found to be appropriate to use the linear approximation for the present systems and then, assuming counterion exchange to proceed much more rapidly than relaxation, we have [14]

$$R_1 = p_o R_o + \Sigma\, p_{Bi}\,\frac{2\pi^2}{5}\,\chi_i^2\,\tau_{ci}\left[\frac{0.8}{1+4\omega^2\tau_{ci}^2} + \frac{0.2}{1+\omega^2\tau_{ci}^2}\right] \quad (1)$$

Table 1
$^{23}Na^+$ transverse relaxation rates, R_1 and R_2, in a solution (1.64 g) containing 0.2 molal dermatan-4-sulfate and 0.3 molal Na^+ as a function of added HCl

HCl, 1 M (μl)	pH	α	R_1 (s^{-1})	R_2 (s^{-1})	p_0	$\Delta (T_2/T_1)$	τ_c (ns)
0	12.44	1.6	64.1	71.6	0.4 –1	0.863–0.884	1.9 –1.8
110	8.33	1.0	65.4	69.5	0.4 –1	0.922–0.935	1.35–1.25
140	4.10	0.83	45.5	49.3	0.5 –1	0.883–0.907	1.7 –1.5
170	3.43	0.67	33.6	38.1	0.7 –1	0.788–0.828	2.6 –2.2
200	2.59	0.50	25.2	30.3	0.85–1	0.624–0.683	4.1 –3.5
260	1.35	(0.17)	24.5	29.0	0.90–1	0.634–0.657	4.0 –3.8

See text for definition of quantities and for description of the calculation of τ_c

$$R_2 = p_0 R_0 + \Sigma p_{Bi} \frac{\pi^2}{5} \chi_i^2 \tau_{ci} \left[0.6 + \frac{1}{1+\omega^2\tau_{ci}^2} + \frac{0.4}{1+4\omega^2\tau_{ci}^2} \right] \quad (2)$$

Here, p_0 is the fraction of free counterions, these having a relaxation rate R_0 ($R_{10} = R_{20}$), and p_{Bi} the fraction bound at a site i on the polymer. ω is the angular NMR frequency.

The points, denoted (i) and (ii), will be considered in some detail for dermatan sulfate and in table 1 results are presented of direct relaxation time measurements for different neutralization degrees. As can be inferred from eq. (1) and eq. (2), a comparison between R_1 and R_2 gives τ_c, provided p_0 can be estimated. In the present case, even a broad variation of p_0 has only a small influence on the calculated τ_c value. It is interesting to note (table 1) that τ_c varies appreciably with the degree of neutralization, pointing to a conformational transition in the α-range where the carboxylic acid groups start to become deprotonated. The exact nature of the conformational transition cannot be adequately discussed at the present stage of investigation.

On the basis of these τ_c values (interpolated when necessary), we may calculate the quantity $p_B\chi^2$ for different α-values. (For simplicity we assume a single type of binding site; more precisely the calculated values of $p_B\chi^2$ are weighted averages over the different classes of sites.) From fig.2 it can be inferred that $p_B\chi^2$ increases strongly in the α-range 0.6–0.9, while outside this range only small changes are obtained. Using different reasonable counterion binding expressions to estimate p_B, the quadrupole coupling constant was deduced. χ is found to be essentially independent of α, with a value in the vicinity of 150 kHz. This value was used in combination with the line-widths from the FT spectra to estimate p_B. As can be inferred from fig.3, the obtained variation of p_B with α is qualitatively according to expectation. (Choosing χ 200 kHz instead seems less appropriate, since it leads to much lower p_B values for high α values.)

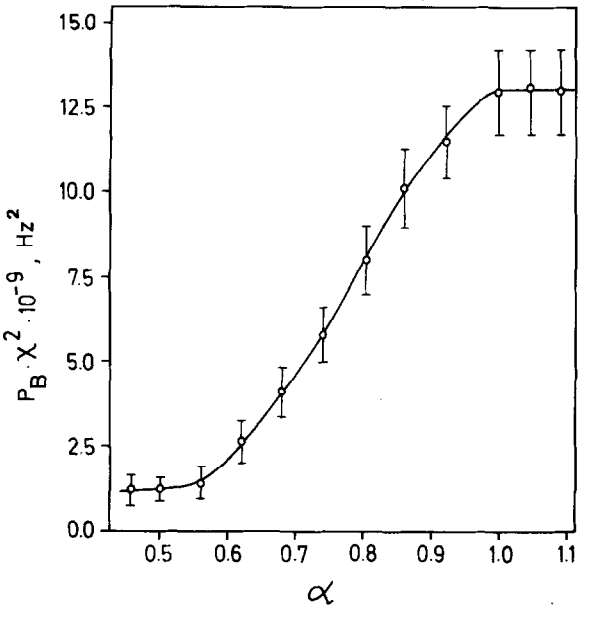

Fig.2. The quantity $p_B\chi^2$ (cf. text) as a function of α for sodium ions bound to dermatan-4-sulfate. The values are obtained from the R_2 values given in fig.1 and with τ_c values from table 1, interpolated when necessary.

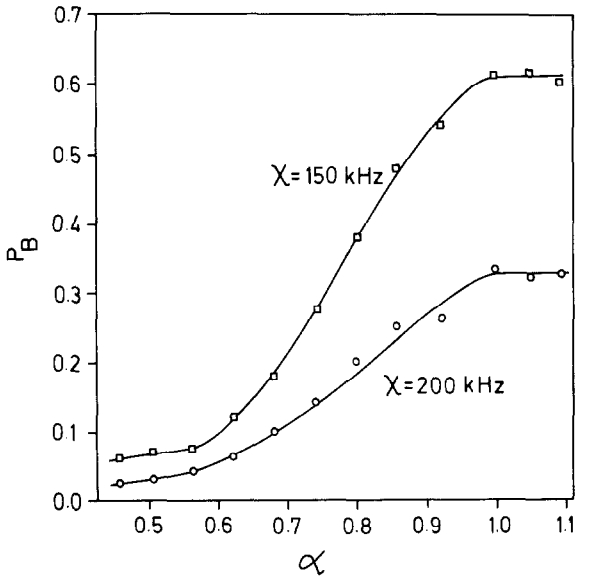

Fig.3. The fraction of bound counterions, p_B, in dermatan-4-sulfate as a function of α. The values were deduced from $p_B\chi^2$ of fig.2 with the two quadrupole coupling constants 150 kHz (□) and 200 kHz (○), respectively.

It seems that in this analysis we have obtained a consistent picture demonstrating the general applicability of the procedure. We have been able to deduce information on counterion binding, correlation times and quadrupole coupling constants. Certainly, these quantities contain significant information on the systems, but a discussion should be deferred until we have the corresponding information for other systems as well.

Acknowledgement

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