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Binding of Metal Ions to Polysaccharides. III. Partial Molar Volumes of Chondroitin Sulphate Systems

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Determination of partial molar volumes provides information on the solvation of species in solution. As such this quantity is an obvious tool to distinguish between specific and atmospheric binding. We found that in aqueous systems of chondroitin sulphate and copper(II) ions specific binding effects play an important role. Binding to only one charged group (carboxylate or sulphate) could be ruled out on the basis of our results. However, a distinction between possible modes of binding to two charged groups could not be made merely from the partial molar volumes. Nevertheless, for the binding of divalent metal ions the involvement of two carboxylate groups, or one carboxylate group and one sulphate group, appears most likely.

Introduction

Recent developments in the technique of density measurements [1] have resulted in a large number of studies on molar volumes of simple ions in solution [2]. Studies by Conway *et al.* [3] and Inagaki and Teramoto [4] around 1960 indicated a peculiar volumetric behaviour for polyelectrolyte metal ion systems. Soon afterwards, Strauss and Leung [5] developed a method to describe the interactions between metal ions and polyelectrolytes in terms of site binding. They used tetraalkylammonium ions, which supposedly do not bind specifically. Tondre and Zanà [6] developed these ideas further and critically examined the experimental technique. Skerjanc [7] and Boyd [8] discussed the behaviour of polyelectrolyte solutions at low concentrations (10^{-2}–10^{-3} mol dm^{-3}). The resulting partial molar volumes at infinite dilution are generally interpreted by means of the cell theory developed by Katchalsky and Eisenberg [9] or by the simpler condensation theory of Manning [10]: the binding of counter ions is dependent on the charge density of the polyion in the sense that condensation of ions on the polyion takes place up to a certain charge density. In earlier parts of this series we explored the possibilities of site binding in the polysaccharide chondroitin sulphate, ChS†, (Fig. 1), by means of polyelectrolyte catalysis [11] and spectrometric and potentiometric measurements at high pH [12]. We are interested in chondroitin sulphates because these polysaccharides play a role in the transformation of cartilage to bone, in which their calcium binding properties might be important [13, 14]. In this article we describe the volumetric properties of aqueous solutions of chondroitin sulphate A in the presence of copper(II), sodium(I), tetramethylammonium (TMA^+) and 1,2-bis(trimethylammonium)ethane (N^{2+}) cations to obtain additional insight into the interactions of chondroitin sulphate with metal ions in solution, especially with regard to site binding.

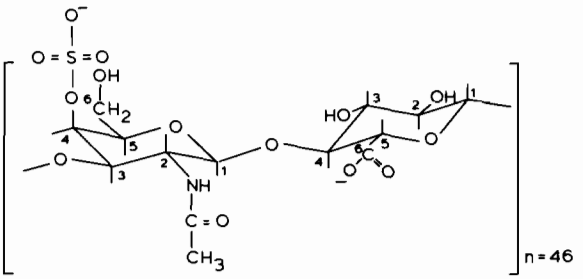

Fig. 1. Structure of chondroitin sulphate A. In chondroitin sulphate C the sulphate group is present in the 6-position and n = 69.

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†The systematic name for ChSA is: (1 → 4)-O-β-D-glucopyranosylic acid, (1 → 3)-2-acetamido-2-deoxy-β-D-galactopyranose-4-sulphate (or -6-sulphate for ChSC).

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Recently, Tsuge *et al.* [15] published volumetric measurements of chondroitin sulphate A with some non-transition metal ions. The present study supplements these results with data on the binding of copper(II) and on the binding of copper(II) and sodium(I) to desulphated chondroitin sulphate (Ch).

Experimental

Materials

For most materials we refer to reference 11. In the present investigation only chondroitin sulphate A was used. Solutions of ChSA with metal ions were prepared by means of the ion exchange technique described by Tondre and Zana [6]. In this method the sodium salt of chondroitin sulphate is transformed into the acidified form on an ion exchange column, Dowex 50W-H^+, and subsequently reacted with metal hydroxide. $Cu(OH)_2$ was prepared according to the method of Weiser *et al.* [16]. TMAOH (a solution of 10% in water) was obtained from Merck.

The molecular weight of Na_2ChS was determined by potentiometric titration of the acidified form with sodium hydroxide in a glove box under nitrogen atmosphere. Na_2ChS was found to contain 1½ mol H_2O, NaCh 3½ mol H_2O per mol of substance. All calculations were corrected for this. The dried material is very hygroscopic.

Density Measurements

The molar volumes were evaluated from density measurements carried out with an Anton Paar model DMA 02 D densimeter. The temperature of the thermostat was 298.15 ± 0.004 K, which corresponds to a reproducibility of the oscillation time of ±2 ppm. Since all measurements were done relative to water at 298.15 K (d_o = 0.997047 g cm^{-3} [17]), the instrument was calibrated with aqueous NaCl solutions in the concentration range 0–2 mol dm^{-3}, using the equation

$$\Delta d = d - d_o = K(\tau^2 - \tau_o^2) \qquad (1)$$

where d is the solution density, τ the oscillation period and K a constant [1]. With the densities of the NaCl solutions calculated from accurate literature data [18], the value of K was determined as (1.688 ± 0.003) × 10^{-11} g cm^{-3} s^{-2}.

Results

The apparent molar volume ϕ_v as a function of the density is given by

$$\phi_v = \frac{M}{d_o} - \frac{(d - d_o)10^3}{c d_o} \qquad (2)$$

in which M is the molecular weight of the solute, d_o the density of the pure solvent, d the density of the solution and c the concentration of the solute (in the present case we followed the usual convention of moles of disaccharide units dm^{-3}).

Extrapolation of ϕ_v to infinite dilution provides the partial molar volume of the solute $\overline{V^o}$. For a discussion of this extrapolation we refer to references 6, 7, 8 and 19. A complication is the fact that when site binding occurs, no simple relation between ϕ_v and c can be expected. Because we observed only a very small concentration dependence of ϕ_v in the range 10^{-1}–10^{-2} mol dm^{-3} we did not extrapolate to c = 0, but used the average of at least five values of ϕ_v. It follows from equation (2) that the molecular weight has to be known to calculate ϕ_v. This quantity was computed for the system $ChSA^{2-}/r_M M^{n+}/(2 - nr_M)TMA^+$ ($r_M = [M^{n+}]/[Ch(S)]$) as follows:

$$M_{wsyst} = M_w(ChSA^{2-}) + r_M M_w(M^{n+}) + (2 - nr_M)M_w(TMA^+) \qquad (3)$$

For chondroitin the calculations are analogous. The system is then: $Ch^-/r_M M^{n+}/(1 - nr_M)TMA^+$ so that

$$M_{wsyst} = M_w(Ch^-) + r_M M_w(M^{n+}) + (1 - nr_M)M_w(TMA^+) \qquad (4)$$

The values of $\overline{V^o}$ determined by us are listed in Table I.

Discussion

Central to the discussion of binding of metal ions to polyelectrolytes is the fact that at infinite dilution, unlike in simple electrolyte systems, dissociation of cation and anion is not complete [10]. The question remains then: how are the counter ions bound to the polyion? It is generally agreed upon that a distinction can be made between site binding, in which the counter ion is localized on the polyion, and atmospheric binding in which mobile counter ions are trapped in the electrostatic field of the polyion [20]. For the analysis of our volume data we used this model.

The measured values of $\overline{V^o}$ are interpreted in the manner of Tondre and Zana [6]. For this the partial molar volumes of the cations are needed. We used the values based on $\overline{V}^o_{H^+} = -5.5$ cm^3 mol^{-1} [2] which leads to values of $\overline{V^o}$ for $Na^+ = -6.7$, $Cu^{2+} = -36.52$ [21], $TMA^+ = 84.2$ [2] and $N^{2+} = 134.1$ cm^3 mol^{-1}. The last value was experimentally determined with an aqueous solution of 1,2-bis(trimethylammonium)ethane perchlorate using $\overline{V}^o_{ClO_4^-} = 50.6$

TABLE I. Partial Molar Volumes in $cm^3\ mol^{-1}$ of Chondroitin Sulphate A and Chondroitin in the Presence of Different Counter Ions.

Polyion (P)	Metal ion (M)	r_M [b]	$\bar{V}^o$ [c]	$(\bar{V}_P^o)_M$ [c]	$\bar{V}_P^o$ [c]	$\delta\bar{V}_{PM}$ [d]
ChS I[a]	TMA^+	2.0	398.3	229.9	229.9	0[e]
ChS II[a]	TMA^+	2.0	396.4	228.0	228.0	0[e]
ChS II	N^{2+}	1.0	360.8	226.7	228.0	−1
ChS I	Na^+	2.0	234.2	247.6	229.9	+18
ChS I	Cu^{2+}	1.0	225.0	261.5	229.9	+32
ChS I	Cu^{2+}	0.5	324.7	258.8	229.9	+29
ChS II	Cu^{2+}	0.2	371.7	244.3	228.0	+16
Ch	TMA^+	1.0	327.4	243.2	243.2	0[e]
Ch	Cu^{2+}	0.2	298.0	254.8	243.2	+12

[a]Different samples. [b]r_M is number of metal ions per disaccharide unit. [c]Estimated error ±1 $cm^3\ mol^{-1}$. [d]$\delta\bar{V}_{PM} = (\bar{V}_P^o)_M - \bar{V}_P^o$, estimated error ±2 $cm^3\ mol^{-1}$. [e]By definition.

$cm^3\ mol^{-1}$ [22]. One can then obtain the apparent molar volume of the polyion in the presence of M, $(\bar{V}_P^o)_M$ by making use of the equation

$$\bar{V}^o = \bar{V}_M^o + (\bar{V}_P^o)_M \tag{5}$$

The explicit mentioning of M in $(\bar{V}_P^o)_M$ expresses the fact that a fraction of the cation condensates on the polyion at infinite dilution.

For ChSA, in which sulphate and carboxylate groups are present, the partial molar volume of the disaccharide unit $(\bar{V}_P^o)_M$ can be calculated as:

$$(\bar{V}_P^o)_M = \bar{V}^o - r_M\bar{V}_M^o - (2 - nr_M)\bar{V}_{TMA}^o \tag{6}$$

and by

$$(\bar{V}_P^o)_M = \bar{V}^o - r_M\bar{V}_M^o - (1 - nr_M)\bar{V}_{TMA}^o \tag{7}$$

for Ch, in which the only charged groups are carboxylate groups. Strauss and Leung [5] postulated that tetramethylammonium ions showed only atmospheric binding. In this assumption the value of $(\bar{V}_P^o)_{TMA}$ equals the partial molar volume of the fully ionized polyion, $\bar{V}_P^o$. Following Tondre and Zana [6], we assume that the hydrophobic contributions to the partial molar volume in the fully ionized and partially ionized states are equal. The site binding effects are then derived from the differences of the system under consideration from the TMA system. In the case of partial molar volumes the quantity $\delta\bar{V}_{PM}$, the change in volume of the polyion, caused by site binding of M, is defined by

$$\delta\bar{V}_{PM} = (\bar{V}_P^o)_M - \bar{V}_P^o = \\ = (\bar{V}_P^o)_M - (\bar{V}_{ChSA(TMA)_2}^o - 2\bar{V}_{TMA}^o) \tag{8}$$

for ChSA, and by

$$\delta\bar{V}_{PM} = (\bar{V}_P^o)_M - \bar{V}_P^o = (\bar{V}_P^o)_M - (\bar{V}_{ChTMA}^o - \bar{V}_{TMA}^o) \tag{9}$$

for Ch.

The calculated values of $\delta\bar{V}_{PM}$ are given in the last column of Table I.

Our finding that $\delta\bar{V}_{PM}$ for N^{2+} is close to zero ($-1\ cm^3\ mol^{-1}$) implies that N^{2+} is a good reference divalent cation, as was also found in our polyelectrolyte catalysis studies [11].

At $r_M = 2$ for Na^+ and $r_M = 1$ in the case of Cu^{2+}, the large positive values of $\delta\bar{V}_{PM}$ indicate appreciable site binding. The value of $\delta\bar{V}_{PM} = 18\ cm^3\ mol^{-1}$ for Na_2ChSA is in reasonable agreement with the value of $\delta\bar{V}_{PM} = 23.7\ cm^3\ mol^{-1}$ found by Tsuge *et al.* [15]. These authors found a value of $\delta\bar{V}_{PM} = 35.1\ cm^3\ mol^{-1}$ for BaChSA and a value of $\delta\bar{V}_{PM} = 32.7\ cm^3\ mol^{-1}$ for CaChSA, which are close to our value for CuChSA. The differences emphasize the important role of the charge of the cation in binding to polyelectrolytes. The absolute value for $\bar{V}_{(TMA)_2ChSA}^o$ ($261.6\ cm^3\ mol^{-1}$) measured by Tsuge *et al.* [15] is considerably higher than our value of $229\ cm^3\ mol^{-1}$. As for $\delta\bar{V}_{PM}$ the difference from our value is much smaller, we suppose that the discrepancy in the absolute value is due to the neglect of the water content of Na_2ChSA by Tsuge *et al.* Table I shows that $\delta\bar{V}_{PM}$ becomes larger with increasing r_{Cu}, but shows a saturation effect. $\delta\bar{V}_{PM}$ for Ch is only slightly smaller than $\delta\bar{V}_{PM}$ for ChSA at $r_{Cu} = 0.2$. This observation indicates little influence of the sulphate group on the binding of copper(II) ions. In the literature there is no agreement about the contributions of the carboxylate and sulphate groups to the binding of metal ions to ChS: Mathews [23], Dunstone [24] and Preston *et al.* [25] consider

TABLE II. Calculated Fraction of Copper(II) Ions Bound per Disaccharide Unit (β_M) for the Models I–V (see text for definition of models).

Complex	$\delta\bar{V}_{PM}$	β_M, I	β_M, II	β_M, III	β_M, IV	β_M, V
CuChS	32	0.46	0.53	0.35	0.64	0.78
$Cu_{0.5}TMA_{1.0}ChS$	29	0.84	0.97	0.64	0.59	0.72
$Cu_{0.2}TMA_{1.6}ChS$	16	1.19	1.37	0.91	0.82	1.01
$Cu_{0.2}TMA_{0.6}Ch$	12	–	0.98	–	–	0.72

the binding of, respectively, $[Co(NH_3)_6]^{3+}$, alkaline earth ions and sodium(I), as non-specific. The carboxylate as well as the sulphate group contributes to the electrostatic interaction in their opinion. Gilbert and Meyers, however, describe the binding of calcium(II), lanthanum(III) and yttrium(III) to chondroitin sulphate as specific to the carboxylate group [26]. Tivant *et al.* [27] deduce both electrostatic and site binding of cobalt(II) to the sulphate group from electrical mobility measurements and NMR spectra. However, they do not discuss the role of the carboxylate group at all. Furthermore their writing '$ChSO_4$–Na' and '$ChSO_4$–$N(Me)_4$' suggests only one charged group. Tsuge *et al.* conclude from density measurements that alkaline earth ions bind to the carboxylate groups [15], while Tanaka infers from conductivity measurements that the association of calcium(II) ions with the glycosaminoglucuronan cannot be ascribed solely to electrostatic forces [28], but that specific interactions occur.

We conclude from our density measurements that specific interaction of chondroitin sulphate occurs with copper(II). The small difference between $\delta\bar{V}_{PM}$ for ChS and Ch at $r_{Cu} = 0.2$ indicates site binding of copper(II) to the carboxylate groups in every case. In the next section some arguments will be presented to show that interaction with the sulphate group cannot be excluded completely.

Fraction of Specifically Bound Counterions

Tondre and Zana [6] calculated from $\delta\bar{V}_{PM}$ the fraction β_M = number of bound cations/maximum number of cations that can be bound.

They supposed that upon site binding a certain volume of solvent is released from the electrostriction shells of counter ions and sites of the ligand. The volume which is actually released upon binding cannot be inferred from volumetric measurements alone as it depends on the degree of occupancy of the sites. However, it is possible to calculate the maximum volume of water which can be released. The electrostriction volumes of the charged groups of the polyelectrolytes and those of the counterions are known. For each model of binding (*i.e.* considering the role of the binding sites on the polyanion) the maximum total electrostriction volume released $[\bar{V}^o_{\epsilon(tot)}]$ can be computed. The ratio $\delta\bar{V}_{PM}/\bar{V}^o_{\epsilon(tot)}$ is then the fraction β_M of counter ions that at most are involved in specific interactions:

$$\bar{V}^o_{\epsilon(tot)} = f(\text{binding model}) = \bar{V}^o_{\epsilon}(\text{sites}) + \bar{V}^o_{\epsilon}(M) \quad (10)$$

The term $\bar{V}^o_{\epsilon}$(sites) was determined by Gekko and Noguchi [31] as $\bar{V}^o_{\epsilon}(OSO_3^-) = -30.3$ and $\bar{V}^o_{\epsilon}(COO^-) = -21.2$ cm^3 mol^{-1} from measurements with partially substituted dextransulphates and carboxymethyldextrans, respectively.

$\bar{V}^o_{\epsilon}(M)$ can be calculated from the partial molar volume of the counter ion and the intrinsic molar volume of the solvated ion, $\bar{V}_{intr}$:

$$\bar{V}^o(M) = \bar{V}_{intr} + \bar{V}^o_{\epsilon}(M) \quad (11)$$

For $\bar{V}_{intr}$ the approximation by Mukerjee [22] was used:

$$\bar{V}_{intr} = 2.51\ r^3\ (1 + K)^3 \text{ cm}^3 \text{ mol}^{-1} \quad (12)$$

in which r is the Pauling radius [32] and (1 + K) is the ratio of the radius of the solvated ion r_s and r. We used Mukerjee's value of K = 0.213 for all ions, resulting in $\bar{V}^o_{\epsilon}(Na^+) = -10.54$ cm^3 mol^{-1}. The value of $\bar{V}^o_{\epsilon}(Cu^{2+}) = -38.19$ cm^3 mol^{-1} deviates from a value recently calculated by Lo Surdo and Millero [21] of –40.48 cm^3 mol^{-1}. We suspect however that these authors used the Pauling radius of Cu^+ instead of that of Cu^{2+}.

In the following, five models are compared, in which site binding occurs at the following positions:

I OSO_3^-

II COO^-

III COO^- and OSO_3^-

IV OSO_3^- and OSO_3^- } binding of one M^{n+} to two

V COO^- and COO^- } disaccharide units.

TABLE III. Calculated Fraction of Sodium(I) Ions Bound per Disaccharide Unit (β_M) for the Models I–III (see text for definition of models).

$\delta\bar{V}_{PM}$	β_M, OSO_3^-	β_M, COO^-	β_M, COO^- and OSO_3^-
18	0.43	0.56	0.24

With these models values of β_M were calculated. Because $\bar{V}^o_{\epsilon(tot)}$ is a maximum value, β_M is a minimum. In order to be physically realistic, β_M should of course not exceed one. Table II indicates that two values of β_M calculated with the models I and II (with one binding site) do not fulfil this condition. Thus the specific binding of copper(II) to ChSA involves two charged groups on the polyion, according to this procedure.

Next it was tried to discriminate between the models III, IV and V in the following way.

The limiting law of Manning [10] gives a theoretical value of

$$\beta_{M,condensation} \equiv \beta_{M,atmosphere\,plus\,site}$$

at infinite dilution, so a maximum value of $\beta_{M,site}$ (here β_M). Furthermore, it seems more realistic to assume that $[Cu(H_2O)_6]^{2+}$ loses fewer than six water molecules upon binding (which increases β_M relative to the case of the loss of all six water molecules). It is possible then to calculate a maximum value of $\delta\bar{V}_{PM} = \beta_M \cdot \bar{V}^o_{\epsilon(tot)}$ for the three models. This does not give a means of discrimination because all experimental values of $\delta\bar{V}_{PM}$ were smaller than the theoretical maximum ones. Arguments can be presented to prefer models III and V over model IV. Firstly, binding of copper(II) to sulphate groups alone is not as probable as binding to two carboxylate groups or one carboxylate and one sulphate group. For example, stability constants of the interactions between Cu^{2+} and $ROSO_3^-$ compounds are not known [33], probably because these interactions are too small. Secondly, $\delta\bar{V}_{PM}$ of Ch is nearly equal to $\delta\bar{V}_{PM}$ of ChS. Because Ch has only carboxylate groups, it is likely that at least one carboxylate group is involved in binding. Contribution of the sulphate groups remains possible; chondroitin can be 'forced' to bind to carboxylate groups because there are no sulphate groups.

The models III and V agree with an X-ray diffraction study of calcium chondroitin sulphate A (which has a similar value of $\delta\bar{V}_{PM}$ as copper chondroitin sulphate A [15]) by Cael *et al.* [34]. They found binding at two oxygens of two different carboxylate groups and at one oxygen of a sulphate group. This model was established by NMR measurements on binding of lanthanide ions to ChS [35]. For sodium, binding at OSO_3^-, or at COO^-, or at COO^- and at OSO_3^-, can be postulated. The values of β_M are given in Table III. Discrimination between these models is not possible. An X-ray diffraction study of Na_2-ChSA by Winter *et al.* [36] indicates that direct binding (both sodium and ligand group lose electrostricted water) does not occur.

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Appendix

Calculations of $\bar{V}^o$ for $ChSA^{2-}$ and Ch^- from Group Contributions

Recently, Zana [19] showed that partial molar volumes of polymers can be approximated from group contributions obtained from measurements on small molecules. For uncharged polymers the calculated values for $\bar{V}^o$ agree very well with the experimental ones. For polyelectrolytes, however, the calculated values are always larger than the experimental ones. Our analysis along the same lines gives the following results: using $\bar{V}^o_{lactose}$ = 207.6 cm^3 mol^{-1} [29] and for the other groups Zana's values, we calculated $\bar{V}^o_{ChS^{2-}}$ = 241.0 cm^3 mol^{-1}. In an alternative procedure using $\bar{V}^o_{glucuronate}$ = 102.4 cm^3 mol^{-1} [30] and $\bar{V}^o_{galactose}$ = 111.9 cm^3 mol^{-1} [29], a value of 239.8 cm^3 mol^{-1} was obtained. Our experimental average value of 229 cm^3 mol^{-1} for $ChSA^{2-}$ is qualitatively in agreement with the findings of Zana for other polyelectrolytes that $\bar{V}^o_{exp} - \bar{V}^o_{calc} < 0$ because of cooperative electrostriction. The magnitude of this difference, 11 cm^3 mol^{-1}, is somewhat smaller than the sum of the difference for polyacrylate (9.8 cm^3 mol^{-1}) plus that for polystyrenesulphonate (5.7 cm^3 mol^{-1}): 15.5 cm^3 mol^{-1}. This would be expected from the larger distance between charged groups in ChSA. However, for desulphated ChSC we calculate an average value of $\bar{V}^o_{calc}$ = 217 cm^3 mol^{-1}. This value is appreciably lower than $\bar{V}^o_{exp}$ of 243 cm^3 mol^{-1}. We have no explanation for this observation.

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