Inorganica Chimica Acta, 55 (1981) 109–115
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Binding of Metal Ions to Polysaccharides
I. Polyelectrolyte Catalysis by Chondroitin Sulphate

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Received September 26, 1980

The acceleration of the Hg(II) induced aquation of $Co(NH_3)_5X^{2+}$ (X = Cl, Br) by the mucopolysaccharides chondroitin sulphate A and C was studied as a function of the concentration of polyion and Hg(II), of pH and of the ionic strength. The inhibition of this process by a number of divalent ions emphasizes the electrostatic character of the metal-polyion interactions when sulphate groups are involved.

A two phase model justified extrapolation of catalytic properties from the well studied densely charged synthetic polyelectrolytes to the compounds studied here which have a relatively low charge density. Corrections are proposed for the influence of the oligomeric nature of the chondroitin sulphates on the rates. The covalent interactions of Hg(II) with the carboxylic acid groups are discussed.

Introduction

Acceleration of ionic reactions by synthetic polyelectrolytes has been the subject of a large number of investigations in the past decade. Several models have been proposed for the large effects that were observed [1–4]. The term catalysis for this phenomenon has been criticized [4], on the grounds that polyelectrolytes are not true catalysts as they may influence the position of the equilibrium for the reactions. Little, however, has been published about the rate enhancing properties of polyelectrolytes that occur in living systems: one example is Ise's study on the effect of polynucleotides on the mercury(II) induced aquation of bromopentammine cobalt(III) [5]:

$$Co(NH_3)_5Br^{2+} + Hg^{2+} \rightarrow Co(NH_3)_5H_2O^{3+} + HgBr^{+} \quad (1)$$

As part of our investigations into the binding of transition metal ions with natural polychelates [6] we studied reaction 1 in the presence of the mucopolysaccharide (or glycosaminoglycan) chondroitin sulphate*. We expected that the rate enhancements could serve as a sensitive probe for metal ion-polyion interactions. In further parts of this series we shall report about these interactions by means of spectroscopic, viscometric, potentiometric and other techniques. Chondroitin sulphates (Fig. 1) occur, usually linked with proteins, in connective tissues and play a role in the transformation of cartilage to bone, in which their calcium binding properties might be important [7]. For essentially electrostatic phenomena like polyelectrolyte catalysis the important functional groups are the carboxylate group (*cf.* glucuronic acid) and the sulphate group, which occurs mainly in the 4-position in chondroitin sulphate type A (ChSA) and mainly in the 6-position in chondroitin sulphate type C (ChSC). Also, ChSC has a higher molecular weight than ChSA. Protonation of the glucuronate group provides the opportunity to vary the charge density on the polyelectrolyte: the average distance b (as projected on the polymer axis) between the charges can be varied in this way from 0.5 to 1.0

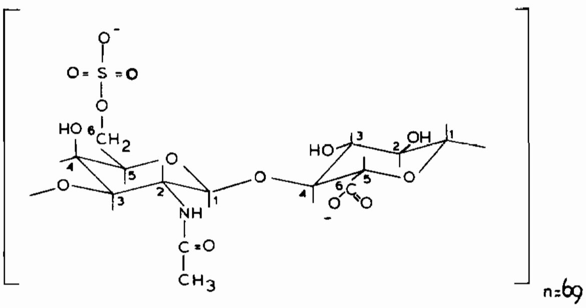

Fig. 1. Structure of chondroitin sulphate C. In chondroitin sulphate A the sulphate group is present in the 4-position.

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*The abbreviation ChS will be used. 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).

nm [8]. A number of questions arise about the physicochemical properties of ChS as expressed in its catalytic action:

1. Can these be described by the same models as the more densily charged (b $\approx$ 0.25 nm) vinylic polymers like polyethylene sulphonate?
2. Are any differences between ChSA and ChSC due to their different chemical structures and/or to their different molecular weights?
3. Can the binding of metal ions to the polyion be described by purely electrovalent interactions, or are covalent interactions present?

In the first case 'catalysis' can be described in terms of the real surface potential ψ of the polyanion, in the second case specific interactions with cations should be contained in ψ.

Experimental

Materials

Chondroitin sulphates were obtained from Sigma as di-sodium salts: ChSA was isolated from whale cartilage, ChSC from shark cartilage. The hygroscopic materials were stored over P_2O_5 *in vacuo* and gave satisfactory analyses for Na, C, H and N. The weight averaged molecular weights $\bar{M}_w$ were estimated by viscometry as 2.9×10^4 for ChSA [9] and 4.3×10^4 for ChSC [8]. The number averaged molecular weight $\bar{M}_n$ was estimated as $\bar{M}_n = 0.8\ \bar{M}_w$ [10] giving $n_A = 46$ and $n_C = 69$. With ^{13}C NMR spectroscopy we could confirm observations [11] that ChSA contains about 15% sulphate in the 6-position and ChSC about 30% sulphate in the 4-position, most likely in the form of a copolymer. Mercury(II) perchlorate was obtained from Ventron (Alpha Division) as the tri-hydrate and its purity was checked according to Flaschka [12]. The cobalt complexes were prepared by the method of Diehl *et al.* [13]. The other metal perchlorates were Fluka analytical grade. Desulphation of ChSC to chondroitin was accomplished with the procedure of Kantor and Schubert [14].

Polyacrylic acids (PAA) with molecular weights stated as 2000, 5000 and 90000 were obtained in the form of concentrated aqueous solutions from Aldrich. Small amounts of insoluble material were filtered off and the equivalent concentrations of PAA determined in a nitrogen filled glove box by titration with sodiumhydroxide. Sodium dextran sulphate (molecular weight 40000) with 2.35 sulphate groups per glucose unit as determined from its Na^+ content was obtained from Sigma. 1,2-bis(trimethylammonium) ethane perchlorate $((CH_3)_3\overset{+}{N}CH_2CH_2\overset{+}{N}(CH_3)_3)(ClO_4)_2$ was prepared from N,N,N′,N′-tetramethyldiaminoethane (Merck) by methylation with methyliodide and precipitation of the product with excess perchloric acid.

Kinetics

The kinetics of disappearance of the halopentammine cobalt(III) species were measured with a Zeiss PMQII Spectrophotometer equipped with a rapid mixing device as described by Balt and Renkema [15]; the aquation of $Co(NH_3)_5Br^{2+}$ was followed at 253 nm, that of $Co(NH_3)_5Cl^{2+}$ at 229 nm. However, below 240 nm appreciable absorption by the polymer occurred and therefore the bromo complex was studied in more detail. Each measurement was done at least in triplo. If not referred to otherwise, the standard conditions of the experiments were:

$[Co(NH_3)_5X^{2+}]_o = 1.0 \times 10^{-5}$ mol dm^{-3}
$[Hg^{2+}]_o = 1.0 \times 10^{-4}$ mol dm^{-3}
$[ChS] = 1.0 \times 10^{-3}$ moles of disaccharide dm^{-3}
$[HClO_4] = 1.0 \times 10^{-2}$ mol dm^{-3}
$T = 298.1 \pm 0.1$ K

The ionic strength I was adjusted with $NaClO_4$, usually to 0.020 mol dm^{-3}. The assumption was made that each charged group and its Na^+ counterion contributed to I as 1:1 electrolytes [16]. I = 0.02 mol dm^{-3} was chosen as a compromise between an ill-defined system at lower I and appreciable competition of Na^+ with the reagents for sites on the polymer at higher I. We estimate that the selectivity for M^{2+} over M^+ is about 100. Essentially pseudo first order kinetics in $Co(NH_3)_5X^{2+}$ were obeyed up to at least two half times according to

$$-\frac{d[Co(NH_3)_5X^{2+}]}{dt} = k_1[Co(NH_3)_5X^{2+}] = = k_2[Hg^{2+}][Co(NH_3)_5X^{2+}] \quad (1)$$

Ishikawa [2] estimates for the reaction in the absence of polyelectrolytes at zero ionic strength $k_{2,0}$ = 2.2 dm³ mol^{-1} s^{-1} for X = Br. We chose this as the reference state, because it represents the bare test ions Hg^{2+} and $Co(NH_3)_5Br^{2+}$. The ratio between the observed k_2 and $k_{2,0}$ then describes the interaction between the bare test ion and the polyion screened by counterions.

Results

The acceleration of reaction 1 by ChSC increased with increasing polyelectrolyte concentration up to [ChSC] = 2×10^{-3} mol dm^{-3} and decreased more steeply at higher polyelectrolyte concentrations (Fig. 2). The concentration dependence was essentially the same for the bromopentammine and the chloropentammine complex: $k_{Br} \approx 200\ k_{Cl}$ at all [ChS]. A series of recycling experiments was performed, in which the reaction mixture after dilution and adjustment to the proper conditions of $[Hg^{2+}]$ and

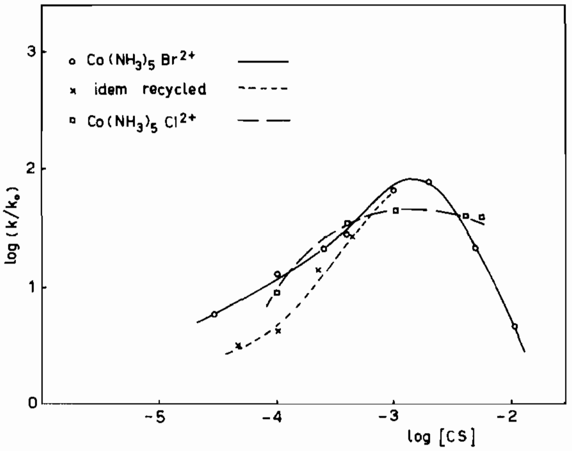

Fig. 2. Polyanion catalysis of the reaction of $Co(NH_3)_5X^{2+}$ with Hg^{2+}. Dependence of the catalytic effect on polyion concentration. $[Co(NH_3)_5X^{2+}] = 1.0 \times 10^{-5}$ mol dm^{-3}; $[Hg^{2+}] = 1.0 \times 10^{-4}$ mol dm^{-3}; $[HClO_4] = 1.0 \times 10^{-2}$ mol dm^{-3}; $I = 0.02$ mol dm^{-3}; $T = 298.1$ K. ○: X = Br; □: X = Cl; ×: recycling experiment with X = Br.

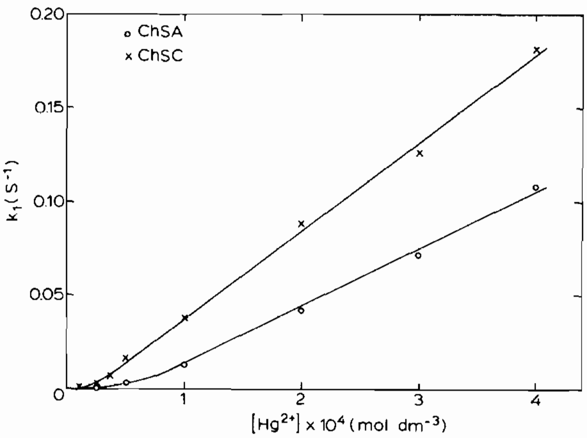

Fig. 3. Dependence of the ChS-catalyzed equation of $Co(NH_3)_5Br^{2+}$ on Hg^{2+} concentration. $[ChS] = 1.0 \times 10^{-3}$ mol dm^{-3}, other concentrations as in Fig. 2.

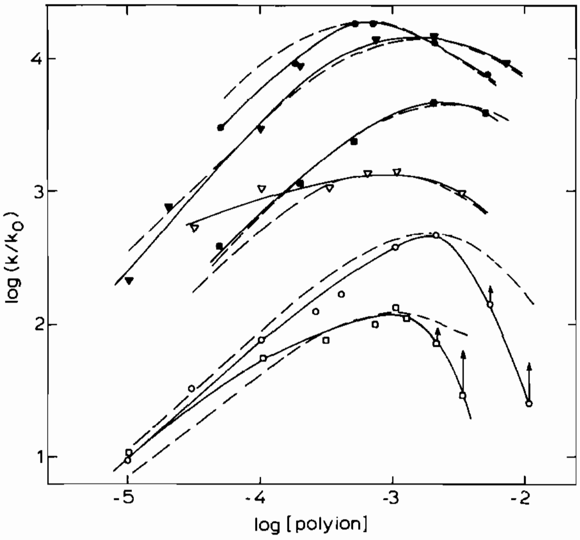

Fig. 4. Polyanion catalysis of the reaction of $Co(NH_3)_5Br^{2+}$ with Hg^{2+}. Dependence of the catalytic effect on polyion concentration at different pH and ionic strengths. Dotted lines are theoretical curves calculated from the maximum in the catalysis from the two phase model. ○: ChSC, pH = 2.0, $I = 0.02$ mol dm^{-3}; □: ChSA, pH = 2.0, $I = 0.02$ mol dm^{-3}; ∇: ChSC, pH = 3.0 (corrected for $HgOH^+$), $I = 0.02$ mol dm^{-3}. The arrows indicate the increase in $\log(k/k_o)$ if ChSA and ChSC were infinitely long polymers (see Appendix B). From Ise en Matsuda [24] at pH = 2.0, $I = 0.014$ mol dm^{-3}, $Co(NII_3)_5Br = 6.0 \times 10^{-5}$ mol dm^{-3}, $Hg^{2+} = 5.0 \times 10^{-4}$ mol dm^{-3}: ●: PSS, ▼: PES, ■: PP.

$[NaClO_4]$ was again used as a catalyst. Appreciable reduction of the rate per cycle pointed at the irreversible poisoning of the catalyst by reaction products. Actually, the decrease in activity was larger than could be explained by neutralization of three charges on the polyion by the product $Co(NH_3)_5 \cdot H_2O^{3+}$. The first order rate constant k_2 increased with increasing [Hg(II)], at least when excess of ChS was present (Fig. 3). In all cases the acceleration by ChSC was larger than that by ChSA (see also Fig. 4). It was found that a certain minimal amount of Hg^{2+}, which was larger for ChSA than for ChSC, was necessary to let the reaction proceed.

The measured acceleration appeared to be fairly independent of pH in the range $2 < pH < 5$. This is due, however, to two opposing factors upon increase of pH: (1) the dissociation of the carboxylic acid group (which for polyions is itself pH dependent): the higher charge density will cause an increase in the rate; (2) the hydrolysis of Hg^{2+} to $HgOH^+$ (pK = 3.52 [18]). This species is expected to be inactive in the induction of the aquation. Addition of halide ions led to a proportional decrease in the rates due to inactive HgX^+ species. Consequently the rate constants for $pH > 2$ have been corrected through multiplication by $(1 + 10^{pH-3.52})$ and thus they are less accurate. The rates decreased sharply with increasing ionic strength I. From a modified Bjerrum-Brønsted-Christiansen equation [19, 20]

$$\text{Log } k = \text{Log } k(I = 0) + 1.018 z_A z_B I^{1/2}/(1 + I^{1/2}) \qquad (2)$$

values for $z_A z_B$ (the product of the charges of the reacting species) of −9.3 at pH = 2 and of −10.4 at pH = 3 were obtained, while in the absence of polyelectrolyte $z_A z_B$ is equal to 4. In Table I the reduction in rate due to partial replacement of Na^+ by ions of higher valency indicates little specificity among divalent ions at pH = 2. The differences should not be considered significant except that for the Mg^{2+} ion, the ion with the highest charge to radius ratio, the inhibition is smaller. Larger differences were observed

TABLE I. Influence on k_1 by the Partial Replacement of Na^+ by Other Metal Ions M^{n+}. Ionic Strength Adjusted to 0.02 mol dm^{-3} with $NaClO_4$. $[M(ClO_4)_n]$ = [ChS] = 1.0×10^{-3} mol dm^{-3}.

M^{n+}	$k_1(s^{-1})^a$	
	ChSC	ChSA
$(Na^+$	0.038	0.014)
Mg^{2+}	0.036	0.008
Ca^{2+}	0.025	
Ba^{2+}	0.028	
Mn^{2+}	0.026	
Ni^{2+}	0.029	
Cu^{2+}	0.027	0.007
Zn^{2+}	0.028	
N^{2+} b	0.028	
Al^{3+}	0.018	0.005
Yb^{3+}	0.016	
Cr^{3+}	0.011	

aEstimated error ± 0.002 s^{-1}. bN^{2+} = 1,2-bis(trimethylammonium)ethane: $(CH_3)_3\overset{+}{N}CH_2CH_2\overset{+}{N}(CH_3)_3$.

for trivalent cations. Because at concentrations of Fe^{3+}, $Co(NH_3)_6^{3+}$ and $Co(NH_3)_5NO_2^{2+}$ higher than 10^{-3} mol dm^{-3} insoluble complexes were formed with ChS, these systems were not further investigated. Desulphated ChS [14] caused only small accelerations: $k_2/k_{2,0} < 3$, at pH $\leqslant$ 3. Dextran sulphate, on the other hand, catalyzed at least 200 times better than chondroitin sulphate, which is in accordance with its higher charge density: at pH = 2 ChS has one charge per disaccharide unit while DS has two sulphate groups in each glucose ring.

Experiments with polyacrylate were complicated because the pK_a values are higher than for ChS [17]: at degree of dissociation $\alpha = 0.5$ $pK_a = 5.70$ for PAA and $pK_a = 3.80$ for ChS.* Consequently, large corrections are needed due to the presence of $HgOH^+$ in the pH range where measurable catalysis occurs. Experiments with PAA at comparable charge density, degree of dissociation $\alpha = 0.2$ at pH = 4.2, and corrected for the $HgOH^+$ present, gave at least a three times higher acceleration than those with ChSC under the same conditions.

Discussion

Models

Sophisticated models have been developed for polyelectrolyte 'catalysis' [2] and the related micelle 'catalysis' [21]. These are based on the cell model and require extensive numerical calculations, while assumptions, *e.g.* about the size of ions and the dielectric constant ϵ around the polyion, remain necessary. Our observations can be described by a simple two phase model as proposed by Morawetz and Shafer [22], and also used by Martinek *et al.* [23] for micelles. This model also emphasizes deviations in the behaviour of ChS from that of the highly charged polysulphonates.

The approximations of the model are [23] (i) the solution consists of two phases: the polyion with average potential ψ and the bulk phase with potential zero. (ii) A definite distribution of reagents exists between the phases. (iii) Exchange is rapid, *i.e.* the reaction does not affect the distribution equilibrium. (iv) The intrinsic rate constant in the polyelectrolyte phase is the same as in the bulk phase, in other words the acceleration is completely due to the higher concentrations of reagents in the polyion phase. The partition can be described by the dimensionless parameter x

$$x = \exp(-e\psi/kT) \qquad (3)$$

in which e is the electronic charge, k Boltzmann's constant, T the absolute temperature and ψ the (phenomenological) potential which a univalent cation experiences in the polyion domain. The volume fraction occupied by the polyion phase is given by ϕ. The increase in rate with increasing polyelectrolyte concentration is explained by the binding of more reactants near the polyion; its decrease at high polyion concentrations by the dilution of the ions in the polyion phase. Because charged polysaccharides are fairly rigid extended structures [24], the polyion phase can be considered as a cylinder and

$$\phi = 1000\pi r^2 l N_{Av} c_p \qquad (4)$$

with r the effective radius (in m), l the length of the repeating unit (in m), N_{Av} Avogadro's Number and c_p the concentration of repeating units (in mol dm^{-3}). For our reactions, however, it is better to use for ϕ the annular space around the polyion. The width ρ of this annulus can be calculated from the radius R of the polyion itself, which is estimated from structural information, as

$$\rho = (r^2 + R^2)^{1/2} - R \qquad (5)$$

The parameters x and ρ can be obtained (Table II) from the maximum in the rate constant as a function of c_p (see Appendix A). We applied this model to the data by Ishikawa (ref. [2], Table II). Values for ρ are found around 0.10 nm for the highly charged synthetic polyions, comparable to the radius of the Hg^{2+} ion: r = 0.11 nm.

This admittedly crude model indicates that reactions take place very close to the polyion *i.e.* in the Stern layer in analogy with double layer theory. On

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*For polyelectrolytes, because of the cooperative interaction between the charges, the dissociation constant K_a for the abstraction of one proton decreases with increasing degree of dissociation.

TABLE II. Polyion Radii (in nm) Calculated from the Two Phase Model.

Polyion[a]	Radius of cylinder r	Radius of polyion R	Radius annulus ρ	Potential ψ (mV)	
				from k_{max}	from $k = f(Hg^{2+})$
PES[b]	0.21 ± 0.03 [e]	0.25	0.08	−145 ± 15	
PSS[b]	0.36 ± 0.08	0.68	0.09	−137 ± 15	
PMES[b]	0.45	0.80	0.12	−119	
PP[b]	0.20 ± 0.05	0.20	0.08	−144 ± 18	
ChSA	1.4	0.6	0.94	−72	−82
ChSC	1.1[c]	0.8	0.6	−85	−89
	0.9[d]	0.8	0.4	−69	
DS	1.0	0.6	0.6	−140	
		0.8	0.4		

[a] Abbreviations: PES = poly(ethanesulphonate), PSS = poly(styrenesulphonate), PMES = poly(methacryloxyethylsulphonate), PP = polyphosphate, ChS = chondroitinsulphate, DS = dextransulphate. [b] From reference 2. [c] $I = 0.02$ mol dm^{-3}. [d] $I = 0.10$ mol dm^{-3}. [e] Standard deviation of the mean of the results by several workers.

the contrary, for ChSA and ChSC using Tanaka's [8] dimensions (in the latter the sulphate group extends more), we found larger values for ρ, in agreement with the lower potentials and thus the more diffuse character of the double layer around polysaccharides. The values of x and ϕ obtained from the maximal catalysis are used for the calculation of the acceleration at other concentrations of ChS (Fig. 4). Contrary to the case of the synthetic polyions [24], where very good fits were obtained, for ChS significant deviations especially at high concentrations were observed. In Appendix B a statistical contribution from chain length effects is proposed as an explanation: if a chain does not contain at least one irreversibly bound Hg^{2+}, it does not contribute to the effective polyion domain.

Difference between ChSA and ChSC

The oligomer effect explains in part the difference in catalytic properties between ChSA and ChSC ($k_{max}^{ChSC} > k_{max}^{ChSA}$; $C_{p,max}^{ChSC} > C_{p,max}^{ChSA}$), but after this correction there is a larger effect for ChSC than from statistical effects only. In ChSA the sulphate group in the 4-position is closer to the carboxylate group than in ChSC, where it is in the 6-position. In localized binding therefore cations should bind better to ChSA. On the other hand, in ChSC the sulphate groups stick more out from the polymer backbone and could facilitate the access of two reacting large cations over the more buried sulphate group in ChSA. But the most likely factor is that the cooperative effect of the charges of the polyion results in a higher effective potential around ChSC than around ChSA.

The electrostatic potential around ChSA and ChSC was calculated by means of a summation of the potentials caused by the 92 point charges of ChSA and by 138 point charges of ChSC, respectively. It was assumed that the charges are located on a helix as was deduced from X-ray studies on fibres [25]. Counterions were not taken into account and a value for the dielectric constant ϵ of water of 78 was used. At 0.2 nm from a sulphate group, where bound Hg^{2+} is likely situated, in the middle of the polyion this potential was −270 mV for ChSA and −290 mV for ChSC. With counterion binding of Na^+ these values will decrease but complete levelling might not occur.

Modes of binding

The lack of specificity of inhibition by divalent metal ions (Table I) including the 1,2-bis(trimethylammonium)ethane species ('N^{2+}') indicates the interactions to be purely electrostatic as expected for $Co(NH_3)_5X^{2+}$. The special case of Mg^{2+}, which showed the same behaviour at other concentrations and at pH = 3, might be ascribed to the fact that it is so strongly hydrated that no dehydration at all takes place upon interaction with the polyion, *i.e.* no outer sphere complex is formed. For the other ions even a 100 fold excess of M^{2+} over $Co(NH_3)_5X^{2+}$ and a 10 fold excess over Hg^{2+} only decreased the rates by 30%. This again points at non-specific electrostatic interactions whereby enough space for the access of $Co(NH_3)_5X^{2+}$ to the polyion is left open. The potential at distance r from an infinite continuous line charge is proportional to the charge density β [26]:

$$\psi = -(2\beta/\epsilon)K_0(\kappa r)$$

with K_0 the zeroth order modified Bessel function of the second kind [27], and κ the inverse Debye length. The proportionality of ψ with β will remain for any space averaged potential. For ChS at pH = 2 the average charge density is about four times smaller than that of the vinylic polysulphonates, but the calculated potentials (Table II) especially after oligomer correction twice as small. Thus considerable non-electrostatic, covalent contributions necessarily connected with the binding of Hg^{2+}, which increase the effective potential, should be present. Binding to the carboxylic acid group is most probable in view of the high stability constant, log K = 6.13 [28], of Hg^{2+} with acetate, while pK_a of ChS at this pH is of the order of that of glucuronic acid: $pK_a = 3.23$ [29] and displacement of H^+ by Hg^{2+} should occur.

From the linear dependence of k_1 on $[Hg^{2+}]$ (Fig. 3) a value for the potential ψ can also be derived with the two phase model: combination of eqn. (1) with eqn. A1 leads to the expression for the slope in Fig. 3

$$k_1/[Hg^{2+}] = (\phi x^4 + 1 - \phi)/(\phi x^2 + 1 - \phi)^2 \tag{7}$$

The volume fraction ϕ was calculated with eqn. 4 using the values for r from Table II under the assumption that r is independent of c_p. The values for ψ are in reasonable agreement with those calculated from the maximum acceleration, as can be seen from the last two columns of Table II. That a minimum amount of Hg^{2+} is necessary to have the reaction proceed, might arise from competition with Na^+.

The strong interaction of Hg^{2+} with the carboxylic acid group also explains that the catalytic effect is larger with PAA than with ChS at the same charge density. Actually, this makes reactions involving the mercury(II) ion not the most suitable probe for the investigation of the environment of these kind of molecules. Still, polyelectrolyte catalysis can provide useful information about structure and function of ionic biopolymers, even with the aid of simple models like the two phase model.

Appendix A

Calculation of maximum catalysis for the reaction between cations: $A^{z_A^+} + B^{z_B^+} \rightleftarrows [AB]^{z_A^+ + z_B^+} \rightarrow$ products. From reference [22]:

$$k/k_0 = (\phi x^{z_A + z_B} + 1 - \phi)/(\phi x^{z_A} + 1 - \phi)(\phi x^{z_B} + + 1 - \phi) \tag{A1}$$

After differentiation with respect to ϕ for dilute solutions, $\phi \ll 1$, and not too small ψ, *i.e.* $x \gg 1$:

$$\phi_{max} = x^{-\frac{1}{2}(z_A + z_B)} \tag{A2}$$

and

$$(k/k_0)_{max} = x^{z_A}/\{x^{\frac{1}{2}(z_A - z_B)} + 1\}^2 = = \{x^{-z_A/2} + x^{-z_B/2}\}^{-2} \tag{A3}$$

For $z_A = z_B = 2$ this reduces to

$$\phi_{max} = x^{-2} \tag{A4}$$

and

$$(k/k_0)_{max} = x^2/4 \tag{A5}$$

In combination with (3) and (4) these formulae thus give values for ψ and r from information about the maximum acceleration only.

Appendix B

Qualitatively the deviations from the two phase model for ChSA and ChSC can be attributed to their oligomeric character. For example at $[ChSA] = 10^{-2}$ mol dm^{-3} there are $10^{-2}:46 = 2 \times 10^{-4}$ chains dm^{-3}, but initially only 10^{-5} mol dm^{-3} $Co(NH_3)_5Br^{2+}$. Consequently not all chains are occupied by one of more ions of each reagent and formally these unoccupied chains should not be included in the polyion phase, at least if the exchange between chains is slow on the time scale of the reaction.

Effectively then the polyion phase is smaller at high polyion concentrations and the curves in Fig. 4 will decrease more sharply in that region. Also, the maximum in the catalytic activity of ChSA will be shifted more to lower concentrations than that of ChSC. When $[Hg^{2+}]$ is increased, this oligomer effect decreases in importance and the ratio k_1^{ChSC}/k_1^{ChSA} becomes smaller as Fig. 3 shows. Therefore the effective potential for ChSA from the latter method is more negative than that from the former (Table II, last columns). Quantitatively, this effect can be simply described by a homogeneous distribution of an ion M over all chains. The fraction $f_{n,M}$ of polyions with monomer concentration c_p and monodisperse degree of polymerization n occupied by M is

$$f_{n,M} = n[M]/c_p \tag{B1}$$

and the corrected rate constant k_n takes the form

$$k_n = f_{n,M} k_\infty \tag{B2}$$

A statistical distribution, with possibly more than one M per chain, is described by a Poisson distribution [30] in which the assumption is made that one bound ion does not influence the binding of other ions, giving

$$f_{n,M} = 1 - \exp(-n[M]/c_p) \tag{B3}$$

With B1 the catalytic properties should not increase when $c_p > n[M]$ while from B3 a continuing increase is predicted.

In Fig. 5 relation B3 is applied to Ishikawa's data for oligophosphates, the only example of the dependence of polyelectrolyte catalysis on the degree of polymerization which was found in the literature. For the maxima in the catalysis the observed dependence of k_n/k_∞ is closely followed by the calculated $f_{n,Hg}$, which points at irreversible binding of Hg^{2+} plus perhaps a smaller effect from $Co(NH_3)_5Br^{2+}$. In this case an explanation is that the dissociation of $Co(NH_3)_5Br^{2+}$ from the chain is slow, while its diffusion along the chain and its subsequent reaction with the immobilized Hg^{2+} ion are relatively fast. The time which a cobalt complex spends on a chain without Hg^{2+} is then without any effect, and thus the reaction rate is proportional to the fraction of polymers occupied by Hg^{2+}. Our experiments with PAA can

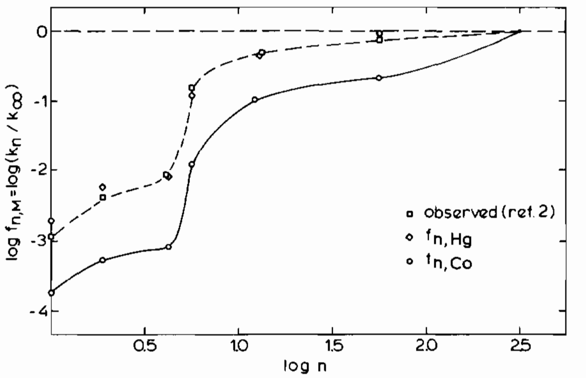

Fig. 5. Acceleration of the reaction of $Co(NH_3)_5Br^{2+}$ with Hg^{2+} as a function of the degree of polymerization of phosphate. □: data by Ishikawa [2]; ◇: calculated for oligomer effect of Hg^{2+}; ○: calculated for oligomer effect of $Co(NH_3)_5Br^{2+}$.

also be described by this model: at [PAA] = 3×10^{-3} mol dm^{-3} $k_{28}:k_{70}:k_{1250}$ = 0.64:0.94:1.00 was found *vs.* calculated 0.65:0.85:1.00. At [PAA] = 1.0×10^{-2} mol dm^{-3} however less difference, 0.76:0.96:1.00 than expected 0.24:0.80:1.00 was found. One explanation is that association between chains [31] is induced by Hg^{2+}. Indeed, turbidity increased rapidly upon addition of Hg^{2+} to PAA solutions and precipitation occurred at lower concentrations than in the case of ChS. For ChSA and ChSC (see arrows in Fig. 4) the oligomer corrections for Hg^{2+} can already account for the largest part of the deviation of the observed rate constant and the two phase model curve. Given the many assumptions underlying the description of this effect, these results are at least encouraging and point again at different modes of binding for Hg^{2+} and $Co(NH_3)_5Br^{2+}$.

Acknowledgements

The author thanks Mr. A. H. van Herk for computational support and Mrs. G. Visser-Luirink, Dr. S. Balt and Dr. M. W. G. de Bolster for stimulating discussions.

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