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Metal ion coordination of macromolecular bioligands: formation of zinc(II) complex of hyaluronic acid

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Abstract

The coordination of zinc(II) ion to hyaluronate (Hya), a natural copolymer, in aqueous solution at pH 6 was investigated by potentiometric and circular dichroism (CD) spectroscopic methods, and by monitoring the changes in macroscopic properties by high-precision measurements. The zinc(II)-selective electrode, and CD measurements proved the binding of zinc(II) by Hya. A number of Hya fragments ($M_r \sim 3.3 \times 10^3 - 1.4 \times 10^6$) were studied to estimate the contributions of the polyelectrolyte effect, the solvation and host–guest interactions to the extra stabilization of the macromolecular zinc(II) complexes as compared with the monomeric unit. The zinc(II) ion activity increase reflected a stability decrease for the fragments with $M_r < 4 \times 10^4$. This molecular weight differs from that where cleavage of the Hya skeleton starts ($\sim 5 \times 10^5$, according to the size-exclusion gel, and anion-exchange chromatographic behavior of the Hya fragments) and from that where the polyelectrolyte effect stops ($\sim 6 \times 10^3$). The excess volumes and Bingham shear yield values of the solutions revealed the transformation of the coherent random coil structure stabilized by intermolecular association in the NaHya to an intramolecular association producing the globular structure of the ZnHya molecule, with a smaller but more strongly bound solvate water sheet.

Keywords: Hyaluronic acid fragments; Zinc(II)-hyaluronate complex; Extra stabilization; Zn-selective electrode; Anion exchange; Size-exclusion gel chromatography

1. Introduction

Macromolecules carrying numerous electron pair donor groups of the same or different kinds are known to bind protons or metal ions significantly more strongly than expected on the basis of their basicity or metal ion affinity.[1] This behavior is well seen by comparing the protonation and metal complexation equilibria of such macromolecules and their fragments of different sizes.[2,3] The extra

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Abbreviations: Hya, hyaluronate; CD, circular dichroism; M_r, molecular weight (Da); SEC, size-exclusion gel chromatography; IEC, anion-exchange chromatography; $\bar{n}$, degree of the complexation, i.e., bound metal:total ligand concentration ratio; V^σ, excess volume; τ_B, Bingham shear yield value; NaHya, sodium salt of Hya; ZnHya, zinc(II) complex of Hya; D, shear gradient; τ, shear stress.

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[†] Professor Kálmán Burger died on the 8th of June, 2000. According to his wishes, the last paper written by him is devoted to Professors Sándor Sonkodi MD and Gyula Baradnay MD, who did their best to save his life.

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stabilization observed may be due to various factors, e.g., (a) the electrostatic effect of the negative charge caused by the donor groups on the macromolecule (polyelectrolyte effect); (b) the lower degree of hydration (solvation) of the donor groups on the macromolecule than in the small molecular analogues, because of the secondary-tertiary structure of the former; or (c) the host–guest interactions between the macromolecule and metal ions, because of the effects of the latter on the structure of the former, etc.

Most macromolecular ligands are biologically active compounds that play important roles in life processes (e.g., proteins, carbohydrates, etc.), behavior which can not be expected for their small molecular fragments. Metal complexation may change their bioactivity, the new properties being utilized in many cases in pharmacological and pharmaceutical practice. This has initiated thorough studies of the coordination chemistry of macromolecular systems, and within this determination of the factors causing the higher stabilities observed for the complexes.

Hyaluronate (Hya) is an alternating copolymer, with repeating disaccharide units as [4)-β-D-Glc*p*A-(1 → 3)-β-D-Glc*pN*Ac-(1 →]$_n$. The average molecular weight ranges from 10^4 to 10^6.[4] It is the structurally simplest, most naturally abundant glycosaminoglycan. It is present as the sodium salt (NaHya) in connective tissues, in the synovial fluid, the vitreous body of the eye, the skin, etc. Its diverse roles in living organisms have been demonstrated by many-sided investigations.[5] It is utilized in different fields of surgery, ophthalmology, dermatology and cosmetics.

Hya has been shown to coordinate to Ca(II),[6,7] Cu(II), Ag(I), Cd(II) and Pb(II) ions,[2,3,8,9] but Parish and Fair[10] stated that it does not bind to zinc(II) ions.

A patent of Burger et al.,[11] however, that led to a new pharmaceutical preparation, proved that in aqueous solution in the pH interval 6.0–6.5, zinc(II) is coordinated by the oxygen-containing donor groups of Hya. Potentiometric measurements with Na^+- and Zn^{2+}-selective electrodes revealed that the hydrolysis of Zn^{2+} and the protonation of Hya limit the pH interval where Zn^{2+} can be coordinated by Hya. Furthermore, as shown by rheological measurements, any electrolyte alters the secondary structure of Hya, in consequence of changes in the charge of this polyelectrolyte, thereby leading to different affinities towards metal ions. Hepes [4-(2-hydroxyethyl)-1-piperazine-ethanesulfonic acid]-NaOH buffer at pH 7.0 (used in Ref. 10) is an electrolyte which has a significant influence on the structure of Hya. This is the reason why gel chromatographic measurements did not disclose the Zn^{2+} binding of Hya, but potentiometric equilibrium measurements in distilled water do prove its existence, as shown in the present paper.

CD spectroscopy has been used to monitor the conformational changes in Hya in solution, due to the presence of organic solvents,[12,13] alterations in pH or temperature[14] and fragmentation.[15] Changes in the Cotton effect in the vacuum UV region (< 210 nm), assigned to π–π* and n–π* transitions of the acetamide groups, reflect the formation or destruction of the ordered structure of Hya in solutions. These results initiated the idea of using CD spectroscopy to follow the structural changes in Hya that accompany Zn(II) coordination.

Potentiometric equilibrium measurements, rheological investigations and CD studies of ZnHya were used to characterize the ZnHya system and to obtain information on the factors influencing the stability of the complex. These investigations, their results and conclusions are presented in the present paper.

2. Experimental

Materials.—NaHya of pharmaceutical purity, $M_r = 1.5–2.0 \times 10^6$ (a product of Chemical Works of Richter Gedeon Ltd., Budapest, Hungary) and ZnHya, prepared according to Ref. 11, served as parent compounds.

Hya fragments of different M_r were prepared by acidic hydrolysis of NaHya solutions (containing 2 mg NaHya/mL distilled water) with HCl at pH ~ 2 and at 343 K. To follow the degradation process, the reaction was stopped at different times and the fragments of different M_r were precipitated with EtOH after adjustment of the pH to 6–7 and the addition of 4% NaCl to the solution. The precipitates were collected on a glass filter, washed first with 80% v/v EtOH to remove excess NaCl and then with anhyd EtOH, and dried at rt in vacuum. Aq solutions of the latter products were used for further investigations.

The molecular weights of the fragments were determined by two chromatographic procedures: (a) size-exclusion (gel) chromatography (SEC) and (b) anion-exchange chromatography (IEC).

Chromatographic procedures.—SEC was performed with a TOSOHAAS GMPWXL column measuring 7.8 mm × 30 cm; 13 μm; MPWXY 3206. Eluent, 0.9% NaCl in distilled water. Flow rate, 0.4 mL/min. Detection, at 210 nm. Injected volume, 100 μL (concentration, 0.1 mg/mL). The standards used for calibration of the gel chromatographic column were:

Polyethyleneoxide, Tosoh Corp.	$M_r = 1{,}000$
Polyethyleneoxide, Tosoh Corp.	$M_r = 46{,}000$
Polyethyleneoxide, Tosoh Corp.	$M_r = 885{,}000$
Sodium hyaluronate Healon Pharmacia	$M_r = 4{,}000{,}000$
Sodium hyaluronate Healon Pharmacia	$M_r = 5{,}000{,}000$

IEC was performed with a PL-SAX 8 μm column, 4000 Å, 50 × 4.6 mm. Flow rate, 1 mL/min., with gradient elution at 313 K. Detection, at 210 nm. Injected volume, 25 μL (concentration, 1 mg/mL).

Eluent A: 20 mM NaCl		Eluent B: A + 350 mM $NaClO_4$	
Gradient A:	0 min	Gradient B:	0 min
	B% = 28		B% = 10
	15 min		15 min
	B% = 43		B% = 55
	17 min		17 min
	B% = 100		B% = 100

Potentiometric equilibrium measurements.—A Zn^{2+}-selective electrode based on *N*-phenyliminodiacetic acid *N′*,*N′*-dicyclohexyl-bis-amide as electrode-active substance,[16] connected to a Precision Radelkis digital millivoltmeter, was used to measure the Zn(II) activity changes during the complex-formation process. A silver | silver chloride electrode served as a reference. The titration cell was thermostated to 298 K, and the pH of the solution was kept at pH 6.0 during titrations. The reproducibility of the measurement was ± 0.2 mV.

Since any salt which may be used to keep the ionic strength of the solution constant has been shown by rheological measurements (viscosity, density, etc., see later) to cause significant changes in the structure of the macromolecules in solution, and since these changes proved to depend on the size of the macromolecule, no salt was used to control the ionic strength. The measurements were performed by titrating standard aq Zn(II) solutions (1.00×10^{-3} M $ZnSO_4$) with aq solutions of NaHya and its fragments with different molecular weights. Each Hya or Hya fragment solution used in these titrations was prepared by appropriate dilution of the compound in question to have the same concentration referred to dimeric Hya units (4.50×10^{-3} M). In this way, the differences between the experimental mV versus mL Hya titration curves reflected the differences caused in the Zn(II) ion activities by the different metal-ion affinities of the Hya fragments of different sizes. A series of typical titration curves is presented in Fig. 1.

Since NaHya served as a parent compound in all measurements, interactions between Hya and Na^+ have also to be taken into consideration. The complex formation process is in practice partly a Zn^{2+}–Na^+ exchange. To study the latter equilibrium, a Na^+-selective glass electrode (Corning Cat. No 476210) was used with the same apparatus as described above.

Circular dichroism (CD) measurements.—The CD spectra were recorded in a Hellma cylindrical cell with 1 mm optical path length on a JASCO J-710 spectropolarimeter in the wavelength range 185–250 nm. A spectral bandwidth of 0.5 nm and a scan rate of 2 nm/min were used. The optical density of the solutions did not exceed 50% of the maximum allowed by the instrument.

Density measurements; excess volume (V^{σ}) determinations.—The densities of solutions of NaHya and ZnHya of different compositions were determined with an Anton-Paar. DMA 58 vibrational densitometer. The accuracy of these measurements (± 0.0001 mg/mL) permitted calculation of V^{σ}[17] of the solutions studied from the density differences of the latter solutions and those of their components, $V^{\sigma} = V_{tot} - V_{solid}$, where V_{tot} is the total volume of the system, and V_{solid} is the volume of the Hya compounds. The concentration dependence of V^{σ} of NaHya and ZnHya and of Hya fragments of different sizes, and the effect of Zn^{2+} coordination by Hya on the above parameter, was determined in aqueous solution with this method. The concentrations of the solutions were in the range 0.05–0.5 g/100 mL.

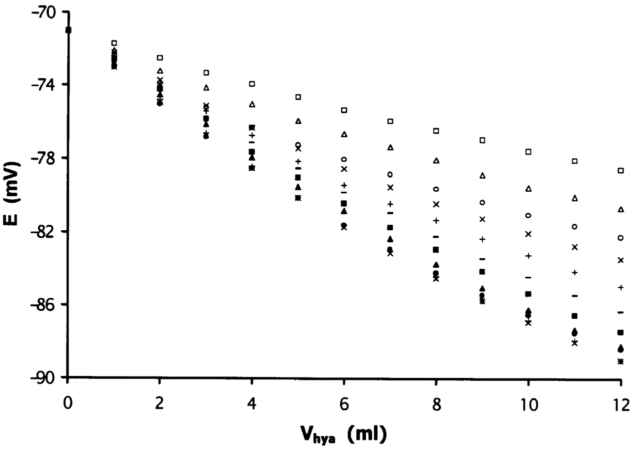

Fig. 1. Titration of Zn(II) ions with standard aqueous solutions of Hya fragments of different M_r values, but the same concentrations (calculated for dimeric units): $c_{Zn^{2+}} = 1.00 \times 10^{-3}$ M, $c_{Hya} = 4.50 \times 10^{-3}$ M. The symbols □, Δ, O, X, +, _ downwards from the upper curve relating to the calibration, performed by titrating the standard Zn(II) solution with bidistilled water, represent the titrations performed with Hya fragments of $M_r = 4.01 \times 10^2$, 3.30×10^3, 6.50×10^3, 1.45×10^4, 4.00×10^4, 1.20×10^5, 2.87×10^5, 4.96×10^5, 8.37×10^5, and 1.43×10^6 Da, respectively.

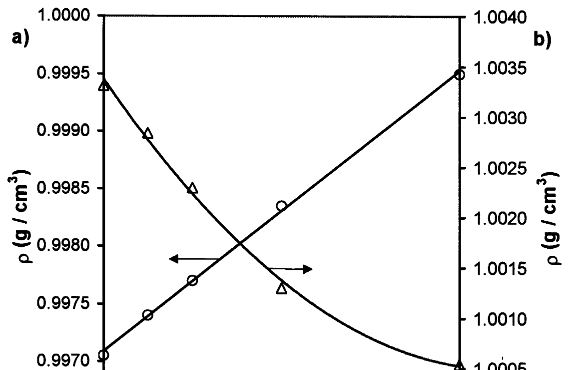

Fig. 2. The concentration dependence of the density of ZnHya in aqueous solution (a) and in isotonic NaCl solution (b).

Viscosity measurements.—The viscosities of aq NaHya and ZnHya solutions of different concentrations were determined with a HAAKE RV 20/CV 100 low-shear rotation viscosimeter at 298 ± 0.1 K in the shear gradient (D) interval 0–100 s^{-1}.

Shear stress values (τ), their dependence on D and Bingham shear-yield values (τ_B) derived by extrapolation of the τ versus D curves[18] and their concentration dependence served as information sources to rationalize the different chemical behavior of the NaHya and ZnHya solutions used in the equilibrium measurements. The concentrations of the solutions were 0.05–0.5 g/100 mL for both Hya compounds.

Measurements in isotonic NaCl solutions.—All measurements reported above were performed in solutions made with bidistilled water as solvent, which did not contain any compound other than the components of the systems investigated.

Since it is known that macromolecules, and especially polyelectrolytes, may undergo structural changes when dissolved in salt solutions, in order to obtain information on the nature of such changes, in the case of Hya and its fragments, the density, excess volume and viscosity measurements in aqueous solutions reported above were repeated in analogous solutions prepared with isotonic (0.15 M) NaCl solution.

1. Density measurements: The densities of both NaHya and ZnHya increased with increasing concentration in aqueous solutions, but decreased in NaCl solutions, as illustrated for ZnHya in Fig. 2.
2. Excess volume determinations: V^{σ} values of both NaHya and ZnHya were found to

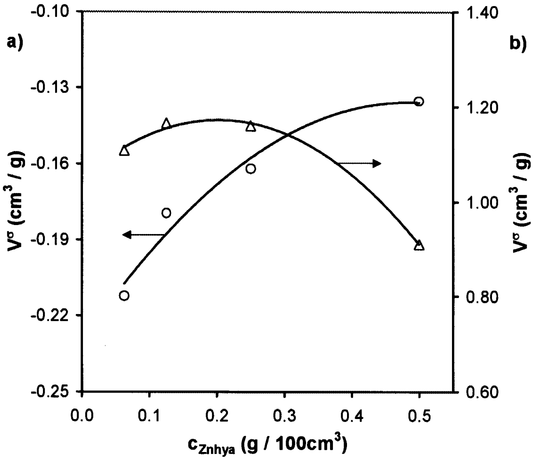

Fig. 3. The concentration dependence of the excess volumes of ZnHya determined in aqueous (a) and isotonic NaCl solutions (b).

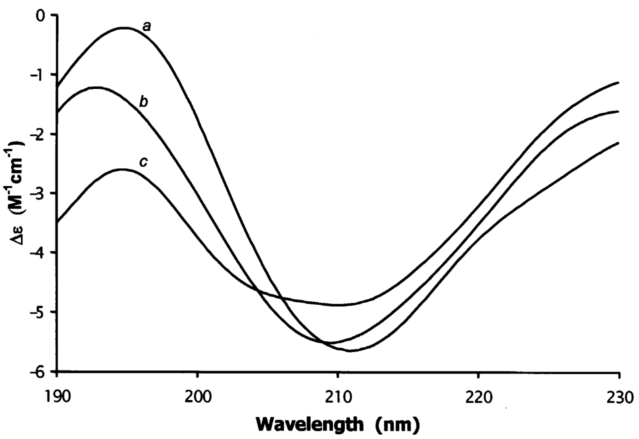

Fig. 4. Molar CD curves of Hya (a) and of solutions containing Hya and Zn^{2+} in concentration ratios of 1:1 (b) and 1:3 (c). The total concentration of Hya was 1.4×10^{-4} M in each case.

have a negative sign in aqueous solutions, but a positive sign in NaCl solutions, indicating a weaker solvation (water coordination) of Hya in the latter than in the former system. Fig. 3 compares the concentration dependence of the excess volumes of ZnHya in aqueous (a) and in isotonic NaCl (b) solutions.

3. The τ versus D curves were similar for both systems, but with significantly lower (~ 50%) τ values for the NaCl solutions.

Comparison of the experimental results in (1), (2) and (3) reflect significant changes in the secondary structure, space requirements and solvation of Hya when dissolved in NaCl solution instead of distilled water.

To separate the effects of the electrolyte and of complex formation on the structure and stability (extra stabilization) of ZnHya, only the results of experiments performed in distilled water were used in the text.

3. Results and discussion

CD measurements.—According to literature data,[12–15] no ordered structure of Hya is expected in aqueous solutions of pH ~ 6 (which were used in all of our reported investigations) if they do not contain organic solvent. This is confirmed by curve (a) in Fig. 4, which resembles those in Ref. 12. The addition of $ZnCl_2$ to this solution, while the Hya concentration and the pH are kept constant, results in small but significant changes in the CD spectra (curves b and c): the intensity of the band at a lower wavelength (π–π* transition) increases with increasing Zn(II) content of the system, clearly indicating the ordering of the Hya structure in aqueous solution due to the coordination of Zn(II) by the macromolecule. In the absence of the metal ions, this type of change could not be demonstrated in our systems.

Chromatographic measurements.—The SEC measurements reflected increase in the retention volumes as a function of the time of decomposition of samples in the time interval studied. With the use of appropriate calibration materials, these changes were used to calculate the M_r of the Hya fragments. The IEC measurements of the same samples indicated a different picture. The retention volumes of samples with M_r between 2×10^6 and 5×10^5 according to SEC, displayed slight changes and only below $M_r = 5 \times 10^5$ they became significant.

In SEC, the retention volume depends on the size of the molecule, including the water bound as solvate layer to the carbohydrate chain. In IEC, the number of anionic groups on the species studied determines the retention volume and consequently, the retention volumes depend on the size of the polyelectrolyte, i.e., on the M_r of the fragment, but not on its solvation.

The different results of the two chromatographic methods (well reflected in Table 1)

Table 1
Size-exclusion gel chromatographic (SEC) and anion-exchange chromatographic (IEC) data on Hya samples from the acidic degradation experiment at pH ~ 2, $T = 343$ K

Degradation time (min)	SEC			IEC	
	Retention volume (mL)	Change of retention volume (%)	M_r (kDa)	Retention volume (mL)	Change of retention volume (%)
0	5.32	0.00	2780	13.78	0.00
30	5.44	2.31	2280	13.67	0.80
60	5.72	7.56	1460	13.65	0.94
120	6.01	13.02	913	13.51	1.96
180	6.19	16.37	686	13.37	2.98
360	6.35	19.38	531	13.29	3.56
1440	6.91	29.84	217	12.17	11.68

Table 2
Size-exclusion gel chromatographic (SEC) and anion-exchange chromatographic (IEC) data on Hya samples dried at 343 K in vacuum

Drying time (min)	Water content (%)	SEC			IEC	
		Retention volume (mL)	Change of retention volume (%)	M_r (kDa)	Retention volume (mL)	Change of retention volume (%)
175	14.87	5.96	0.17	1900	10.89	0.17
210	4.49	6.06	1.51	1570	10.91	0.02
300	2.11	6.29	5.36	988	10.90	0.10
420	1.46	6.48	8.54	688	10.89	0.15
1440	~0	6.92	15.91	294	10.66	2.33

indicate that the procedure used for the cleavage of the macromolecule in the M_r interval between 2×10^6 and 5×10^5 caused the M_r decrease by partial dehydration of NaHya with minor cleavage of the carbohydrate chain. The real decomposition of NaHya starts below $M_r = 5 \times 10^5$.

To confirm the above suggestion, the thermal dehydration of NaHya was performed at 343 K in vacuum, thereby ensuring that the molecule did not undergo cleavage during the drying process. The SEC and IEC results for the dried samples show a similar picture to the degradation experiment (compare Tables 1 and 2). The results also clearly show that the thermal treatment of NaHya leads to an irreversible transformation of the carbohydrate chain, resulting in lower solvation than that of the original NaHya. The same procedure is assumed during the first steps of fragmentation, leading to species with $M_r > 5 \times 10^5$. Therefore, in our comparisons the molecular weights determined by SEC were used.

Potentiometric measurements.—The first evidence of the coordination of Zn(II) by Hya was the potentiometric titration of NaHya in aqueous solution by a standard Zn(II) solution with a Na^+-selective electrode. The corresponding titration curve clearly indicates that in the aqueous solution at the beginning of the titration, ~ 50% of the Na^+ content of the stoichiometric salt NaHya is bound by Hya and that during the titration with the standard Zn(II) solution, Zn(II) ions substitute all the coordinated Na^+ ions, leading to the formation of ZnHya.

Fig. 1 shows a series of potentiometric titration curves where a standard aqueous Zn(II) solution was titrated with aqueous solutions of NaHya and its fragments of different molecular weights by using a Zn^{2+}-selective electrode. Each solution contained the same

Table 3
The apparent Zn(II) complex formation 'constants' (log K values) of Hya and Hya fragments of different M_r, calculated from the individual points of the Zn(II) selective electrode titration curves [a]

$c_{Hya}/c_{Zn^{2+}}$	M_r (Da)					
	401	3300	6500	14,000	63,000	1,430,000
1.34	1.94	2.23	2.26	2.48	2.57	2.75
	(0.06)	(0.10)	(0.11)	(0.16)	(0.18)	(0.23)
1.78	1.93	2.30	2.29	2.51	2.60	2.78
	(0.05)	(0.09)	(0.10)	(0.15)	(0.17)	(0.21)
2.23	1.96	2.21	2.31	2.52	2.63	2.82
	(0.05)	(0.08)	(0.10)	(0.13)	(0.16)	(0.20)
2.67	1.93	2.21	2.35	2.54	2.66	2.84
	(0.04)	(0.08)	(0.09)	(0.13)	(0.15)	(0.18)
3.12	1.92	2.22	2.37	2.57	2.68	2.88
	(0.04)	(0.07)	(0.09)	(0.12)	(0.14)	(0.17)
3.56	1.93	2.22	2.38	2.58	2.70	2.91
	(0.04)	(0.07)	(0.09)	(0.11)	(0.13)	(0.16)
4.01	1.99	2.27	2.39	2.59	2.71	2.92
	(0.04)	(0.07)	(0.08)	(0.11)	(0.12)	(0.15)
4.45	2.01	2.23	2.40	2.60	2.74	2.95
	(0.04)	(0.06)	(0.08)	(0.10)	(0.12)	(0.14)
4.90	2.00	2.23	2.41	2.61	2.76	2.97
	(0.04)	(0.06)	(0.07)	(0.09)	(0.11)	(0.13)
5.34	2.01	2.22	2.42	2.63	2.77	2.99
	(0.04)	(0.05)	(0.07)	(0.09)	(0.10)	(0.13)

[a] The corresponding $\bar{n}$ values are given in parenthesis.

concentration of Hya fragments calculated per dimeric unit of Hya. The electrode potential decrease reflects the decrease in Zn(II) ion activity in the systems (according to the Nernst equation) due to the metal ion coordination of Hya and Hya fragments. The different positions of the curves relating to the fragments of different molecular weights reflect the differences in metal ion affinity of the latter.

Computer evaluation of the data revealed that the systems, except for the smallest fragments ($M_r < 6 \times 10^3$), could not be characterized by the conventionally used complex stability constants. The 'constants' calculated for the different systems[‡] (Table 3) indicated that the affinity of the ligand for the metal ion decreased with increasing complexation state ($\bar{n}$ = bound metal:total ligand concentration ratio), similarly to the protonation behavior of Hya.[19]

Free (uncomplexed) Zn(II) ion concentrations ($[Zn^{2+}]$) were derived from the primary experimental mV values, relating to the same mL Hya volumes of the different titrations performed with solutions of Hya fragments of different M_r (but of the same concentration per dimeric unit of Hya). One of the $[Zn^{2+}]$ versus M_r of the Hya fragment curves at different titration degrees (the ratio of the total metal ion and total ligand concentration, $c_{Zn^{2+}}:c_{Hya}$) is shown in Fig. 5. From this, it can be seen, that at a given $c_{Zn^{2+}}:c_{Hya}$ ratio, $[Zn^{2+}]$ slowly and evenly increases in the titrations with Hya fragments of M_r from 10^6 down to $M_r = 4 \times 10^4$. Below $M_r < 4 \times 10^4$ (at about 2×10^4), the decreased complexation caused a sudden $[Zn^{2+}]$ increase, indicating the disappearance of the extra stabilization of the Zn(II) complexes of Hya fragments. This is appreciably different from the $M_r \sim 5 \times 10^5$, where cleavage of the Hya skeleton becomes significant (the borderline between the dehy-

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[‡] The apparent stability constants were calculated (similarly as in Ref. 3) by means of the equation:

$$\log K = -\log[Zn^{2+}] + \log\frac{\bar{n}}{1-\bar{n}}; \quad \bar{n} = \frac{c_{Zn^{2+}} - [Zn^{2+}]}{c_{hya}}$$

where [ ] and c represent equilibrium and total concentrations, respectively.

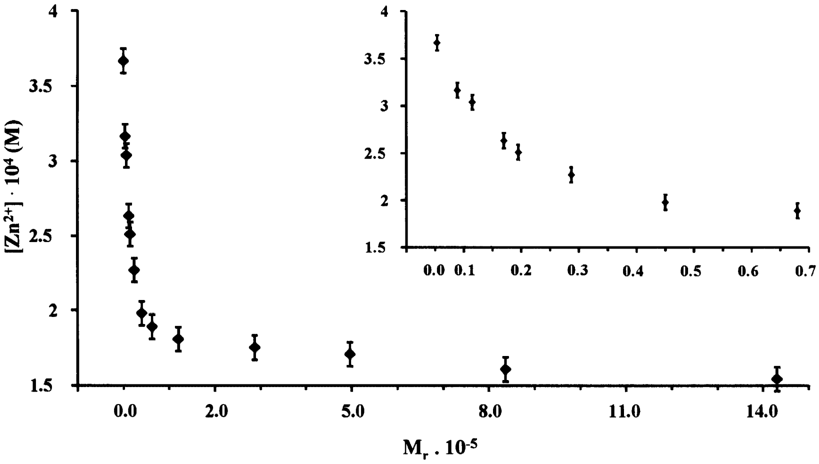

Fig. 5. The free Zn(II) ion concentrations ($[Zn^{2+}]$) plotted as a function of M_r of the Hya fragments. $c_{Zn^{2+}} = 4.55 \times 10^{-4}$ M; $c_{Zn^{2+}}:c_{Hya} = 1:5.34$. The insert shows the enlarged section of the curve down to $M_r < 6 \times 10^4$.

dration and fragmentation processes as indicated by the SEC and IEC results). It does not agree either with $M_r < 6 \times 10^3$ at which the $\bar{n}$ dependence of the equilibrium constants of the ZnHya fragments disappears. It may be concluded from these observations that neither dehydration nor the polyelectrolyte effect of Hya can be considered to be the dominant factor in the extra stabilization of ZnHya.

Since Hya is strongly hydrated and in aqueous solution forms a random coil structure stabilized by hydrogen-bonded three-dimensional clusters of the solution,[20] it may be suggested that stereochemical changes (e.g., host–guest interactions) may play the dominant role in the extra stabilization observed in Zn(II) Hya complex formation. To corroborate this suggestion, in the following the macroscopic properties of the NaHya and ZnHya systems were compared.

Excess volume (*V*$^{\sigma}$) *determinations.*—The V^{σ} values of NaHya and ZnHya solutions, calculated from the precision density measurements on the aqueous solutions and of their components (Figs. 3(a) and 6), proved to have negative signs, indicating that water molecules bound by Hya have a smaller volume in both systems than in bulk water. The significantly higher negative value of V^{σ} for ZnHya than for NaHya points to the stronger solvation of the Zn(II) complex than that of the Na salt. This does not mean that ZnHya binds more water molecules than NaHya. Rheological measurements (see later) indicate just the opposite: ZnHya binds less water, but more covalently than NaHya. In both systems, increase of the concentration of solute (NaHya or ZnHya) caused decreases in the negative V^{σ} values, suggesting that intermolecular association compete with water coordination in the systems.

The effects of the size of the Hya fragments on the water coordination of Hya, were studied in systems containing Hya fragments with $M_r < 4 \times 10^4$ (near the point where the extra stabilization of the Zn(II) complex comes to an end). In contrast with the previous results,

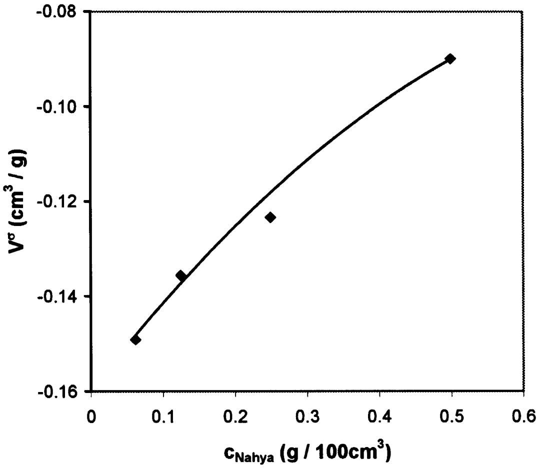

Fig. 6. Concentration dependence of the excess volume of NaHya determined in aqueous solution.

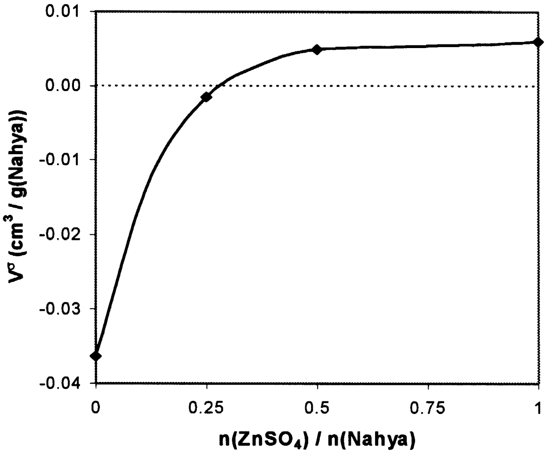

Fig. 7. Effects of the $ZnSO_4$:NaHya concentration ratio on the excess volume of the system for Hya with $M_r = 3 \times 10^4$, the concentration of Hya being kept at a constant value: $c_{Hya} = 0.25$ g/100 cm^3.

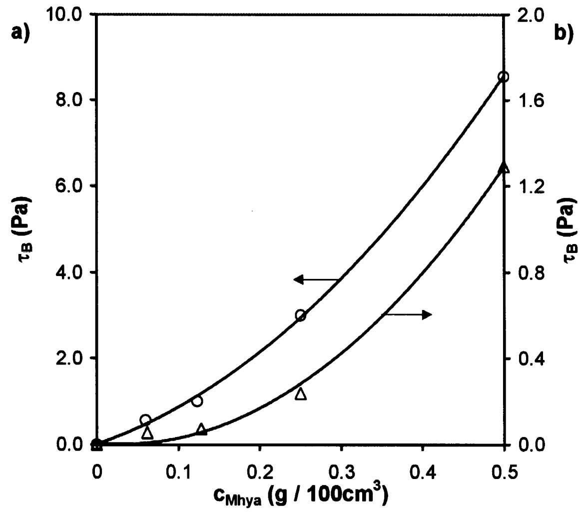

Fig. 8. Concentration dependence of Bingham shear yield (τ_B) values for aqueous solutions of NaHya (a) and ZnHya (b).

the substitution of Na^+ by Zn(II) resulted in increasing V^σ (Fig. 7), indicating that in the complexes where the extra stabilization of the Zn(II) complex was not observed, the stronger binding (lower excess volume) of solvent water by the Zn(II) complex could not be observed.

Viscosity measurements.—The τ versus D curves were used to obtain the τ_B values for aqueous solutions of NaHya and ZnHya. The τ_B values (and their concentration dependence) (Fig. 8(a) and (b)) were ~ six times higher for the NaHya system. This suggests that the coherent random coil structure stabilized by the intermolecular association of molecules in the aqueous solution of NaHya does not appear in the ZnHya solution, i.e., the intermolecular association typical for NaHya is transformed to an intramolecular association due to the substitution process. This is accompanied by transformation of the random coil structure into the significantly smaller globular structure of the macromolecule, with a smaller but more strongly bound solvate water sheath. Latter phenomenon is supported by the fact, that the size of ZnHya, determined by SEC, proved to be smaller than that of the NaHya used for its preparation. However, the IEC retention volumes of the two samples were found to be identical (Fig. 9), showing that the decreased size of ZnHya is due to its lower hydration and not to the lower M_r of the Hya skeleton. This change in hydration was similarly reflected in the compressibility studies of Davies et al.[21]

The above results strongly suggest that the incorporation of Zn(II) in this globular structure can be considered one of the sources of extra stabilization of the Zn(II) complex, besides the polyelectrolyte effect of Hya. This is in agreement with the proposal of the macrochelate type coordination of Hya in Zn(II) complex.[22] At the same time the more strongly bound water could be interpreted in terms of water molecules coordinated to Zn(II) while hydrogen bonded to ligand alcoholic OH groups, similarly as found in other sugar type complexes.[23]

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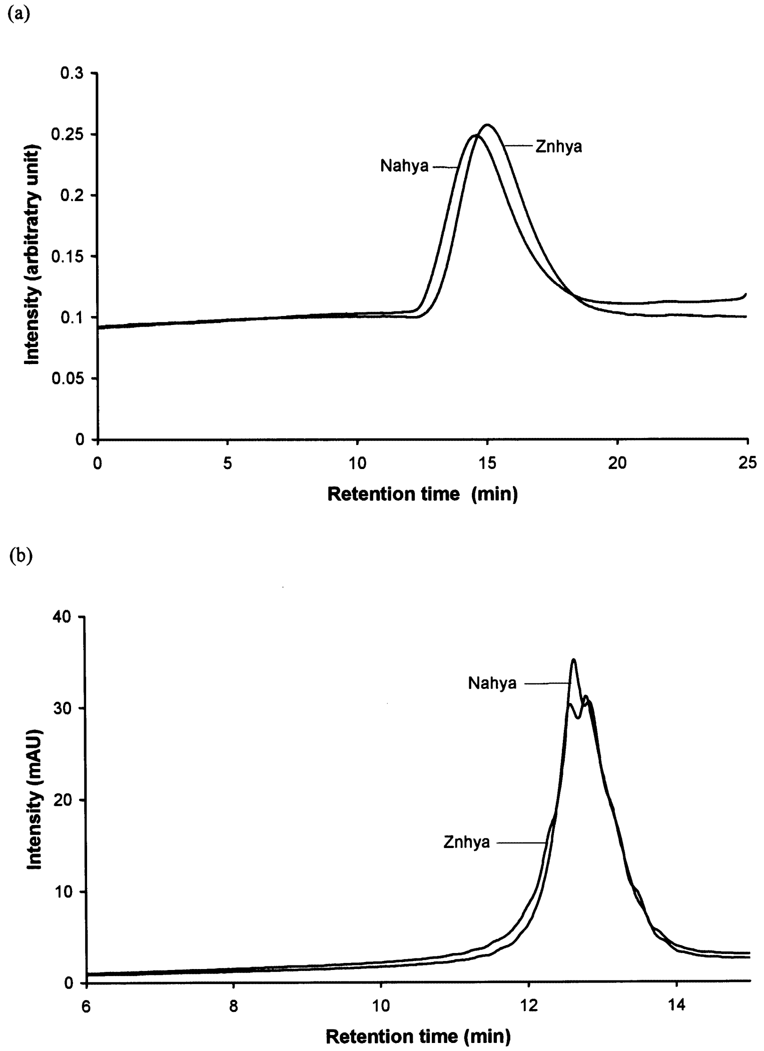

Fig. 9. SEC (a) and IEC chromatograms (b) of NaHya and ZnHya prepared from the same Hya compound. On the basis of the SEC method the M_r of NaHya proved to be larger than that of ZnHya (2.36×10^6 and 1.63×10^6, respectively) for reasons discussed in the text.

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