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^{1}H, ^{7}Li AND ^{133}Cs MULTICOMPONENT SELF-DIFFUSION NMR STUDY ON ION BINDING OF Li^{+} AND Cs^{+} TO NUCLEOTIDES AND AGGREGATION OF NUCLEOTIDES IN AQUEOUS SOLUTION

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The Fourier transform NMR pulsed-gradient spin-echo self-diffusion technique was used for studies of nucleotides (AMP, CMP, GMP and UMP) with Li^{+} or Cs^{+} added, in $^{2}H_2O$. ^{1}H-, ^{7}Li- and ^{133}Cs-NMR-based self-diffusion data on the constituents provide a picture of both the degree of ion binding to nucleotides and the self-association of nucleotides in aqueous solution. Self-diffusion coefficients were investigated in a concentration range up to 0.3 molal nucleotide in $^{2}H_2O$, while keeping the metal ion concentration of Li^{+} or Cs^{+} at twice the nucleotide concentration throughout the investigations. The self-association studies reveal that the aggregation constants of the Li salts differ only slightly from the corresponding constants for the disodium salts of the mononucleotides. Within a two-site bound-free model for the counterions and a cooperative indefinite aggregation model for the nucleotides one finds that the degree of ion binding for all these nucleotide systems remains approximately constant, in spite of increasing aggregate concentration. This corresponds to the well-known polyelectrolyte ion condensation behaviour, indicating that large aggregates are formed, supporting previous findings by the present authors on the aggregation behaviour of nucleotides. An observed large effect on the ^{17}O relaxation of water in nucleotide systems can only be reconciled with the presence of relatively large aggregates in solution.

1. Introduction

Nucleotides, nucleosides and nucleic acid bases are known to self-aggregate in aqueous solution. It is generally accepted from experimental data of numerous techniques [1–36], that the aggregation occurs beyond the dimer stage.

Self-association has also recently been studied using the newly developed Fourier transform NMR pulsed-gradient spin-echo (PGSE) technique for determination of multicomponent self-diffusion coefficients [37–39]. Association phenomena are monitored, as based on time-averaged molecular self-diffusion data, and were previously used in the present field for studies of nucleotide aggregation [28] and on the tendency for certain divalent metal ions to enhance the degree of aggregation [29].

In this work, we have studied the concentration dependence of self-diffusion coefficients of the nucleotides: AMP, CMP, GMP and UMP with Li^{+} and Cs^{+} added. ^{1}H-, ^{7}Li- as well as ^{133}Cs-NMR self-diffusion measurements were performed. By combining the proton diffusion coefficients with the corresponding lithium or cesium diffusion coefficients, it has been possible to determine the degree of ion binding of these metal ions in nucleotide aggregates within a two-site bound-free aggregation model. Nucleotide aggregation itself was simulated in terms of two association models; indefinite aggregation and cooperative indefinite aggregation, as in previous work [29].

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Magnetic relaxation of the water molecules using ^{17}O was measured in the nucleotide systems to check for hydration/aggregate size-related relaxation effects.

2. Experimental

2.1. NMR measurements

The diffusion measurements were performed on a JEOL FX-100 Fourier transform NMR spectrometer equipped with a multinuclear unit and a moving head disc (MHD) based operating system. For the proton self-diffusion studies the 5 mm special proton probe was used. The Li^+ and Cs^+ diffusion measurements were carried out with a 10 mm tuneable multinuclear probe. ^2H_2O was used for internal field/frequency lock. All the measurements were performed at 24.5 ± 0.2°C. The technique entails Fourier transformation of the second half of the pulsed-gradient spin-echo following the second field gradient pulse, keeping the radio-frequency pulse interval (τ) fixed for all durations of the gradient pulses (δ). J modulation and T_2 effects are then constant and need not be considered further. The signal amplitude of a given signal in the spin-echo spectrum is related to the experimental parameters through the relation:

$$A_i \alpha \exp\left(-\gamma^2 G^2 \delta^2 D_i (\Delta - \delta/3)\right) \quad (1)$$

where γ is the magnetogyric ratio of the particular nucleus, G the strength of the applied magnetic field gradient, Δ the interval between the gradient pulses (constant and equal to τ in our measurements) and δ the duration of the applied magnetic field gradient pulses (the notation should not be confused with the identical symbol used for chemical shifts). In the proton diffusion investigations, Δ was kept constant at 140 ms, while δ ranged from 35 to 110 ms at a field gradient strength of approx. 1 G/cm, the exact value of which was calibrated against the proton diffusion of traces of H^2HO in ^2H_2O, as described previously [40]. For the Li and Cs diffusion measurements, Δ was kept constant at 200 ms for Li and 300 ms for Cs, with δ ranging from 40 to 130 ms for Li and from 40 to 260 ms for Cs.

Longitudinal relaxation rates (R_1) for ^{17}O were measured with the inversion recovery method ($\pi - \tau - \pi/2$ pulse sequences) and transverse relaxation rates (R_2) were obtained from the linewidth ($\Delta\nu_{obs}$) at half amplitude of the absorption spectra according to $R_{2,obs} = \pi\Delta\nu_{obs}$. The temperature was 27.4 ± 0.2°C. Proton decoupling was used to eliminate possible T_2 contributions from ^{17}O-exchange broadening around neutral pH [41].

2.2. Materials

The free acid form of the nucleotides AMP (manufacturer's No. A2002), CMP (C1131), GMP (G8252) and UMP (U1752) and the disodium salts (used in the ^{17}O relaxation study) of AMP (A1752), CMP (C1006), GMP (G8377) and UMP (U6375) were obtained from Sigma. Lithium hydroxide, cesium hydroxide and theophylline-7-acetic acid were from Fluka. ^2H_2O, used as solvent, was purchased from Norsk Hydro, Rjukan, Norway.

The nucleotides were used without further purification. Solutions were made up as based on molal concentrations by weight, directly in the 5 or 10 mm NMR tubes from stock solutions of lithium hydroxide and cesium hydroxide in ^2H_2O, in which appropriate amounts of the free acid form of the nucleotides were dissolved so as to obtain a nucleotide concentration of approx. 0.3 molal and at a level of exactly half the hydroxide concentration. The lower concentrations were obtained by successive dilution with ^2H_2O. The pH of the solutions (direct pH readings without correction for the H-^{2}H effect) were checked with a long 3 mm diameter Ingold micro pH-electrode, directly in the NMR tubes, and were for all solutions in the range pH 6.5–7.

Solubility problems arose with GMP in cesium hydroxide. GMP apparently dissolved, but in less than 1 min a white precipitate formed. Due to this problem, it was impossible to measure Cs diffusion in GMP Cs systems.

H_2O enriched to 10 atom% in ^{17}O was obtained from Biogenzia Lemania, Lausanne. 20 μl of this ^{17}O-enriched water was added to the 1 ml solutions of the disodium salts of nucleotides used. Here 5(weight)% ^2H_2O was used for internal lock

and the appropriate molalities were made up by distilled water. The pH was in the range near 7.

3. Measurements and calculational approach

3.1. Models for the self-association of nucleotides

The time-averaged self-diffusion coefficient in a multisite situation is given by:

$$D_{obs} = \sum_i p_i D_i \tag{2}$$

where the diffusion coefficients D_i are mainly dependent on aggregate size, and the fractional contribution of each species is weighted according to its diffusion coefficient. Since diffusion coefficients decrease in a monotonic fashion with aggregate size, the present technique of studying molecular aggregation is necessarily biased to contributions from monomers and lower oligomers, as discussed previously [28,29].

The calculations and the computer simulations on observed diffusion data have been described in previous work [28]. The appropriate corrections for obstruction [42,43] and for hydrodynamic friction [44,45] were made before fitting the experimental data to different association parameters through computer simulations. Details of algorithms and computational procedures will be published elsewhere [46]. As based on previous conclusions [28] two models were considered:

(1) Model 1, indefinite aggregation with all steps equal was simulated with the ASGE11 computer program [28,46].

(2) Model 2, indefinite aggregation, dimerization step unique, i.e., the equilibrium constant for the first (dimerization) step represents an independent parameter. All higher equilibrium constants were set equal (i.e., $K_2 = K_3 = \ldots = K_i$) and were varied independently from the dimerization constant. Influence from all species up to 20-mers was considered in the simulations made by the ASGEIG program [28,46]. A special version of this program, ASGE30, accounts for species up to 30-mers in the case of AMP and GMP at increased concentrations. The numerical values of the fitted equilibrium constants were found in several trial runs to be only moderately sensitive to any physically reasonable span of obstruction correction factors, as in our previous work [28,29].

3.2. Two-site model to describe the ion binding

A two-site model:

$$D_{obs} = p_1 D_1 + p_{agg} D_{agg} \tag{3}$$

for the time-averaged self-diffusion coefficient of counterions successfully describes ion binding/diffusion behaviour in micellar and other polyelectrolyte systems [47,48] and may serve as a useful first approximation to obtain information about ion binding to nucleotide aggregates. Here p_1 is then the fraction of monomers and p_{agg} $(= 1 - p_1)$ the fraction of molecules in aggregates. In analogy with the treatment of diffusion/ion binding data in micellar systems [48] we deduce:

$$C_{agg} = C_{tot} \frac{D_1 - D_{obs}}{D_1 - D_{agg}} \tag{4}$$

where D_{agg} are obtained from eq. 3, using the ^{1}H-NMR diffusion coefficients and the results from the ASGEIG (or ASGE30) simulations for p_1. The curves for D_{agg} vs. total nucleotide concentration were extrapolated to 0.6 molal to obtain necessary values of D_{agg} for the metal ion concentrations above 0.3 molal. D_1 represents the self-diffusion coefficient of monomers in the case of nucleotide diffusion and the self-diffusion coefficient of free counterions in the case of Li^+ or Cs^+ diffusion.

To obtain β, the degree of counterion association, which is the ratio of metal ions and nucleotides in the aggregates, one must account for the different concentration of metal ions and nucleotides in the solutions used. Eq. 4 is therefore rewritten to:

$$\frac{C_{agg}}{C_{tot}} = \frac{D_1 - D_{obs}}{D_1 - D_{agg}} \tag{5}$$

and from this we define β:

$$\beta = \frac{\dfrac{[M^+]_{agg}}{[M^+]_{tot}}}{\dfrac{[XMP]_{agg}}{[XMP]_{tot}}} = \frac{1}{2} \times \frac{[M^+]_{agg}}{[XMP]_{agg}} \tag{6}$$

where $[M^+]_{agg}$ and $[XMP]_{agg}$ are the concentration of metal ions (Li^+ or Cs^+) and nucleotides (X = A, C, G or U), respectively, in aggregates. $[M^+]_{tot}$ and $[XMP]_{tot}$ are the total concentration of metal ions and nucleotides, respectively, where $[M^+]_{tot}$ is $2[XMP]_{tot}$. It should then be clear why a factor of 1/2 enters in the expression for β (eq. 6).

4. Results and discussion

4.1. Association of nucleotides

Tables 1 and 2 summarize the observed diffusion coefficients as obtained by ^{1}H-NMR self-diffusion measurements in the Li^+-nucleotide ^2H_2O systems and Cs^+-nucleotide ^2H_2O systems, respectively. The association constants, K, pertinent to Models 1 and 2 were evaluated from these data. The resulting K values are listed in table 3. As expected, they vary only slightly from the values of K, for the disodium salts of the nucleotides, reported earlier [28]. Model 2 again provides a significantly better agreement between experiment and simulation than Model 1. The criteria were described earlier [29] and the agreement between simulation and experiment is illustrated in fig. 1.

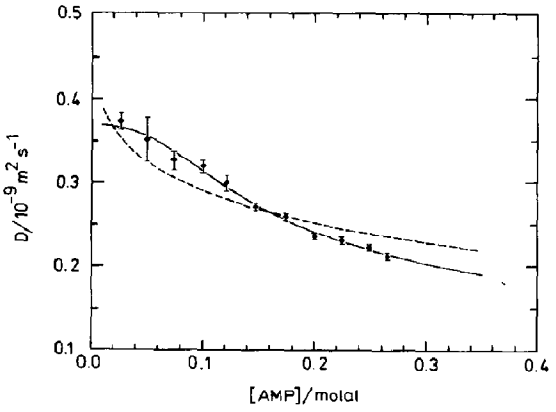

Fig. 1. The concentration dependence of the proton self-diffusion coefficient in AMP Li^+ ^2H_2O systems. Curves are calculated from the fitted parameters for the association Models 1 (- - - - -) and 2 (———).

4.2. ^{17}O Relaxation

Excess spin relaxation rates are given by

$$R_{i,ex} = R_{i,obs} - R_{i,ref} \quad (i = 1,2) \qquad (7)$$

where $R_{i,obs}$ is the relaxation rate observed for nucleotides in 95% H_2O and 5% ^2H_2O (by weight) solutions, and $R_{i,ref}$ the corresponding quantity for the H_2O-^2H_2O solvent at the same tempera-

Table 1

Observed nucleotide self-diffusion coefficients in $Li^+/^2H_2O$ nucleotide systems

Diffusion coefficients are given in units of 10^{-9} m^2 s^{-1}.

AMP		CMP		GMP		UMP	
C (molal)	D_{obs}	C (molal)	D_{obs}	C (molal)	D_{obs}	C (molal)	D_{obs}
0.0249	0.374	0.0253	0.396	0.0248	0.388	0.0256	0.410
0.0489	0.351	0.0477	0.388	0.0492	0.352	0.0705	0.367
0.0736	0.326	0.0718	0.367	0.0744	0.312	0.0944	0.356
0.100	0.319	0.0970	0.355	0.0899	0.316	0.113	0.354
0.121	0.298	0.116	0.341	0.122	0.293	0.145	0.338
0.148	0.270	0.148	0.344	0.149	0.282	0.170	0.342
0.175	0.258	0.172	0.321	0.175	0.256	0.194	0.326
0.201	0.235	0.196	0.315	0.199	0.254	0.218	0.321
0.225	0.230	0.222	0.304	0.220	0.233	0.245	0.294
0.250	0.221	0.248	0.289	0.244	0.228	0.263	0.284
0.266	0.210	0.263	0.291	0.280	0.212	0.301	0.276
				0.302	0.203		

Table 2

Observed nucleotide self-diffusion coefficients in $Cs^+/^2H_2O$ nucleotide systems

Diffusion coefficients are given in units of 10^{-9} m^2 s^{-1}.

AMP		CMP		UMP	
C (molal)	D_{obs}	C (molal)	D_{obs}	C (molal)	D_{obs}
0.0235	0.391	0.0240	0.397	0.0243	0.406
0.0480	0.372	0.0518	0.390	0.0473	0.387
0.0756	0.346	0.0751	0.374	0.0702	0.373
0.102	0.326	0.0958	0.379	0.0935	0.381
0.123	0.318	0.124	0.365	0.126	0.382
0.150	0.301	0.151	0.354	0.169	0.342
0.169	0.280	0.174	0.340	0.201	0.333
0.212	0.279	0.199	0.340	0.224	0.344
0.233	0.267	0.223	0.323	0.248	0.336
0.278	0.248	0.249	0.336	0.274	0.320
0.298	0.240	0.277	0.320	0.300	0.310
		0.300	0.310		

ture. For these systems we found $R_1 \approx R_2$ and the mean of the $R_{1,ex}$ and $R_{2,ex}$ values is plotted in fig. 2. It is obvious that there is a very large effect on the water ^{17}O relaxation upon aggregation. Further, more detailed studies of multifield ^{17}O relaxation along the lines discussed by Halle and co-workers [41,49] could possibly help quantify the aggregation behaviour of nucleotides and in particular the size of the aggregates involved. The ^{17}O relaxation for GMP increases drastically at a concentration of around 0.4 molal. Borzo et al. [31] have reported a similar trend in both ^{23}Na

Table 3

Association parameters pertaining to a data analysis in terms of association Models 1 and 2

K_1, $K_2 \ldots K_n$ and K are the respective fitted association constants according to the association models indicated. D_1 represents the fitted monomer self-diffusion coefficient in units of 10^{-9} m^2 s^{-1}. FOBJ represents the sum of the squares of the residuals in the process of fitting typically 12 data points to the aggregation models (cf. tables 1 and 2).

Cooperative indefinite aggregation				Indefinite aggregation		
K_1 ($molal^{-1}$)	$K_2 \ldots K_n$ ($molal^{-1}$)	D_1	FOBJ	K ($molal^{-1}$)	D_1	FOBJ
Li_2AMP						
0.36	11.04	0.368	0.00428	64.23	0.434	0.05049
Li_2CMP						
2.19	8.29	0.407	0.00223	23.54	0.455	0.00386
Li_2GMP						
1.39	14.69	0.388	0.01062	67.59	0.438	0.04884
Li_2UMP						
1.60	7.74	0.409	0.00773	28.02	0.471	0.00982
Cs_2AMP						
2.43	12.76	0.403	0.00267	67.52	0.486	0.00801
Cs_2CMP						
3.16	5.43	0.410	0.00437	6.93	0.421	0.00465
Cs_2UMP						
1.21	4.55	0.405	0.00631	7.22	0.427	0.00716

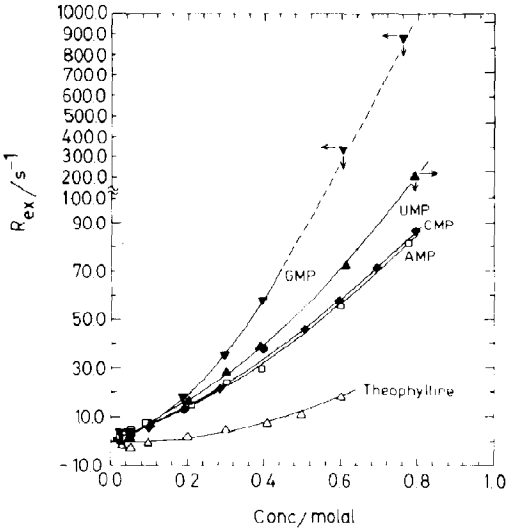

Fig. 2. The concentration dependence of the relaxation rate R_{ex} ($= 1/2(R_{1,ex} + R_{2,ex})$) in nucleotide systems, where the nucleotides, AMP (□), CMP (◆), GMP (▼), UMP (▲) and theophylline-7-acetic acid (△) are solubilized in 5(weight)% ^2H_2O and 95% H_2O with 20 μl ^{17}O-enriched water added to 1 ml of the test solutions. To increase the solubility of theophylline-7-acetic acid it was necessary to add a few drops of concentrated sodium hydroxide solution to these solutions. It was impossible to measure the short T_1 relaxation times for GMP concentrations above 0.4 molal, and the displayed R_{ex} values at 0.6 and 0.8 molal GMP are therefore $R_{2,ex}$.

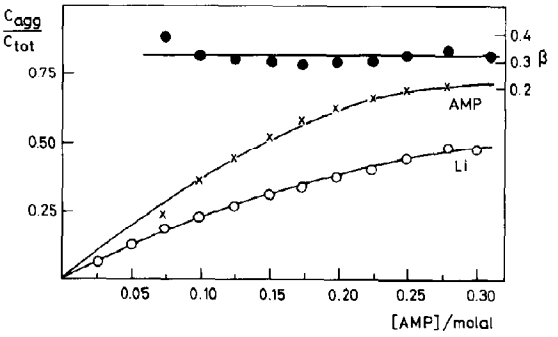

Fig. 3. Ion binding of Li^+ to AMP as a function of concentration. The degree of ion binding, β, is defined in the text (section 3.2). Since C_{agg}/C_{tot} for metal ion and nucleotide is displayed against total nucleotide concentration, the ratio of the two plotted values at a particular concentration is divided by 2 to obtain β.

shift and ^{23}Na-reduced linewidths at a critical concentration of around 0.4 M. This was, by these authors, ascribed predominantly to a $2G_4 \rightleftharpoons G_8$ equilibrium.

4.3. Li^+ and Cs^+ binding to aggregates of nucleotides

Tables 4 and 5 summarize the diffusion coefficients of Li and Cs in metal ion nucleotide sys-

Table 4

Observed Li^+ self-diffusion coefficients in $Li^+/^2H_2O$ nucleotide systems

Diffusion coefficients are given in units of 10^{-9} m^2 s^{-1}.

AMP		CMP		GMP		UMP	
C (molal)	D_{obs}	C (molal)	D_{obs}	C (molal)	D_{obs}	C (molal)	D_{obs}
0.0249	0.0768	0.0253	0.776	0.0248	0.762	0.0256	0.770
0.0500	0.729	0.0506	0.745	0.0730	0.703	0.0514	0.752
0.0737	0.689	0.0746	0.699	0.0970	0.656	0.0753	0.712
0.0985	0.658	0.0995	0.678	0.121	0.637	0.101	0.700
0.124	0.629	0.124	0.652	0.170	0.583	0.126	0.653
0.150	0.599	0.150	0.633	0.196	0.565	0.152	0.625
0.173	0.579	0.175	0.607	0.222	0.536	0.176	0.610
0.198	0.553	0.200	0.585	0.246	0.521	0.200	0.601
0.224	0.533	0.225	0.577	0.275	0.492	0.225	0.573
0.249	0.504	0.250	0.554	0.299	0.476	0.250	0.559
0.278	0.475	0.277	0.539			0.278	0.552
0.300	0.478	0.299	0.517			0.299	0.519

Table 5

Observed Cs^+ self-diffusion coefficients in $Cs^+/^2H_2O$ nucleotide systems

Diffusion coefficients are given in units of 10^{-9} m^2 s^{-1}.

AMP		CMP		UMP	
C (molal)	D_{obs}	C (molal)	D_{obs}	C (molal)	D_{obs}
0.0236	1.544	0.0220	1.548	0.0252	1.558
0.0513	1.454	0.0518	1.487	0.0504	1.503
0.0742	1.422	0.0749	1.459	0.0745	1.441
0.0990	1.365	0.0782	1.422	0.0992	1.405
0.124	1.342	0.0996	1.402	0.125	1.364
0.150	1.281	0.124	1.363	0.151	1.319
0.174	1.241	0.148	1.340	0.174	1.291
0.198	1.184	0.173	1.262	0.248	1.212
0.222	1.171	0.197	1.248	0.274	1.156
0.249	1.132	0.225	1.223	0.300	1.148
0.277	1.118	0.249	1.185		
0.300	1.104	0.276	1.168		
		0.300	1.145		

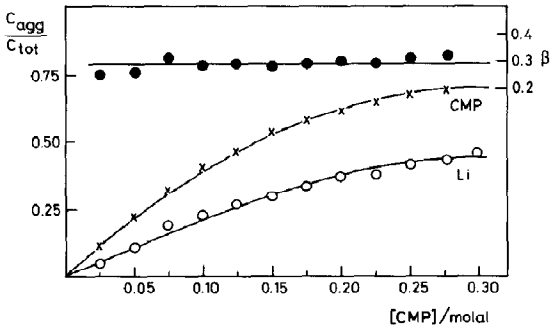

Fig. 4. Ion binding of Li^+ to CMP as a function of concentration; cf. legend to fig. 3.

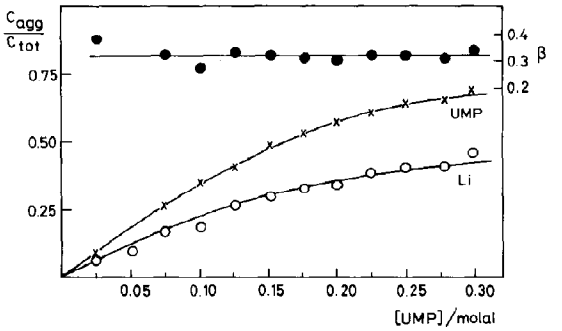

Fig. 6. Ion binding of Li^+ to UMP as a function of concentration; cf. legend to fig. 3.

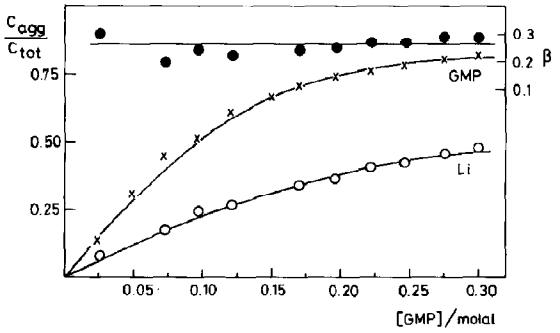

Fig. 5. Ion binding of Li^+ to GMP as a function of concentration; cf. legend to fig. 3.

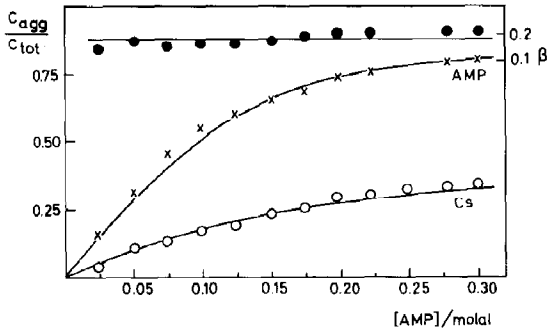

Fig. 7. Ion binding of Cs^+ to AMP as a function of concentration; cf. legend to fig. 3.

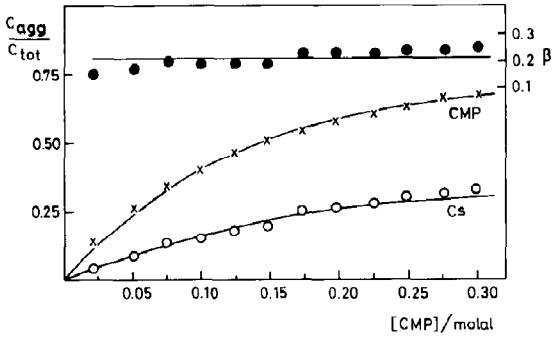

Fig. 8. Ion binding of Cs^+ to CMP as a function of concentration; cf. legend to fig. 3.

tems. The monomer diffusion coefficients, D_1, are found by extrapolation of the D values to zero concentration, since it should be reasonable to anticipate that there will be only monomers and free counterions in the solutions at infinite dilution. $[Li^+]_{agg}/[Li^+]_{tot}$ and $[XMP]_{agg}/[XMP]_{tot}$ are plotted vs. total nucleotide concentration in figs. 3–6. Here it should be kept in mind that the concentration of Li^+ is twice that of the nucleotide concentration throughout (in order to neutralize the XMP so as to make the free acid form of the nucleotides more soluble and obtain a pH of approx. 7). The values for β calculated from eq. 6 are also indicated in the figures. As can be seen, the values of β are approximately the same and independent of the aggregate concentration. The

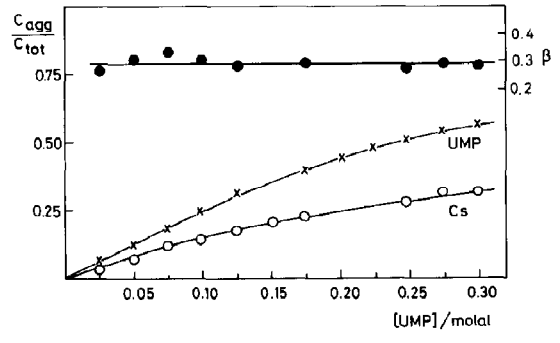

Fig. 9. Ion binding of Cs^+ to UMP as a function of concentration; cf. legend to fig. 3.

same tendency is found in figs. 7–9, which comprise the results with Cs^+ as counterion. The values of β with Cs^+ as counterion are somewhat lower than those obtained for Li^+. This discrepancy could be ascribed to the great difference in size of these two metal ions (cf. ref. 50). One should remember, however, that β, the degree of ion binding, is a semi-quantitative model parameter for describing the continuous ion atmosphere in terms of a two-site bound-free situation. One should therefore not assign too much significance to its actual value or to minor trends with system parameters.

5. Conclusions

A constant degree of ion binding, in spite of increasing aggregate concentration, is characteristic of a polyelectrolyte ion binding behaviour [51–53], indicating that large aggregates are also formed in the present type of aggregation process and supporting our conclusions in previous work [29]. In ref. 28 it was found, for example, that the most probable aggregation number at 0.2 M AMP is about 8 and somewhat lower for the other nucleotides.

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