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A MULTICOMPONENT SELF-DIFFUSION NMR STUDY OF AGGREGATION OF NUCLEOTIDES, NUCLEOSIDES, NUCLEIC ACID BASES AND SOME DERIVATIVES IN AQUEOUS SOLUTION WITH DIVALENT METAL IONS ADDED

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The self-aggregation of the mononucleotides AMP, CMP, and UMP with Mg^{2+} added (nucleotide concentration = Mg^{2+} concentration) up to 0.4 molal or to their solubility limit in ^2H_2O has been monitored through self-diffusion measurements, using the Fourier transform NMR pulsed-gradient spin-echo multicomponent-self-diffusion technique. Also, purine, cytidine, uridine, purine with Mg^{2+} added and both cytidine and uridine with Mg^{2+}, Zn^{2+} or Cd^{2+} added, were studied in the same way. The experimental data were fitted to two different aggregation models. For the mononucleotides with Mg^{2+} added a cooperative indefinite aggregation model, where the first (dimerization) aggregation constant is a magnitude lower than those for the higher aggregation step gives the best agreement between simulations and experiment. Typical values are 0.3 and 12 kg mol^{-1}, respectively. The latter value is about twice that found for the uncomplexed nucleotides. Also, purine and the nucleosides, cytidine and uridine, with divalent metal ions added fit best with this model. The degree of aggregation is increased upon metal ion addition, as previously shown for the mononucleotides. For purine, cytidine and uridine without metal ions added an 'isodesmic', indefinite aggregation model, with the aggregation constant for each step equal, fits the data as well. Here the application of the 'semi-isodesmic' model results in a higher first (dimerization) aggregation constant than is found for the nucleotides. The typical value is 2 kg mol^{-1}. In this case, the evaluated aggregation constants for the higher step become only about twice as large as those of the first step. The same measurements on isopropylcytidine, isopropyluridine and theophylline-7-acetic acid in water show that these three compounds aggregate to the same extent as the nucleosides, cytidine and uridine. Pyrimidine diffusion data reveal no aggregation at all; the application of either model results in essentially zero aggregation constants.

1. Introduction

Nucleic acid bases, nucleosides and nucleotides self-aggregate in aqueous solution. Numerous techniques, such as vapour pressure osmometry, sedimentation equilibria, ultrasound measurements, NMR and other spectroscopic methods [1–34] have been used for studies of this process. The field has been reviewed by Ts'o [35,36] and recent overviews have been given by Sigel et al. [26,27]. It is generally accepted from all the experimental data that the aggregation occurs beyond the dimer stage.

Metal ions play an important role in different biological processes, including those with nucleic acids and their derivatives [26]. Sigel et al. [26,27] have shown by chemical shift-based studies of the protons in both nucleoside 5′-triphosphate and nucleoside 5′-diphosphate in water that the presence of certain divalent metal ions increases the aggregation of these compounds. It was found of

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interest to study the aggregation of mononucleotides, nucleosides and bases, to see if metal ions influence the aggregation of these compounds in a similar fashion.

The method used is based on time-averaged molecular self-diffusion data. The aggregation of a number of nucleotides and of caffeine has recently been studied in this way [37] using the Fourier transform [38] modification pulsed-gradient [39] spin-echo [40] NMR technique (FT-PGSE) as described earlier [41,42]. FT-PGSE techniques have previously been successfully applied for the investigation of aggregation in a number of systems, e.g., in micellar [42–44], microemulsions [44–47], vesicular [48], polymeric polyelectrolyte [49,50] and cyclodextrin solutions [51].

In the present work, we have studied the concentration dependence of the self-diffusion coefficients of purine, cytidine and uridine, and where possible with Mg^{2+}, Zn^{2+} or Cd^{2+} added. The mononucleotides AMP, CMP, and UMP with Mg^{2+} added and isopropylcytidine, isopropyluridine, pyrimidine and theophylline-7-acetic acid have also been investigated. The results have been tested against two association models: indefinite aggregation and cooperative indefinite aggregation. In previous work [37] we have demonstrated that the monomer-single n-mer model is generally inconsistent with observed diffusion data on mononucleotide systems; therefore this model has not been considered in the present investigation.

2. Experimental

2.1. NMR measurements

The diffusion measurements were carried out on a JEOL FX-100 Fourier Transform NMR spectrometer, as previously described [41,42]. ^2H_2O was used for internal field/frequency lock. The technique entails Fourier transformation of the second half of the pulsed-gradient spin-echo following the second field gradient pulse, keeping the radiofrequency pulse interval (τ) fixed for all durations of the gradient pulses (δ). Under these conditions J modulation as well as T_2 effects become constant and need not be further considered. The signal amplitude of a particular signal in the spin-echo spectrum is related to the experimental parameters through the relation

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

where γ is the magnetogyric ratio of the nucleus in question, G the strength of the applied pulsed magnetic field gradient, Δ the interval between the gradient pulses (constant and equal to τ in our studies) and δ the duration of the applied magnetic field gradient pulses (the notation is established in spin-echo NMR and should not be confused with the identical symbol used for proton and carbon chemical shifts). In the present investigation 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. Except for the measurements of CMP and UMP both with Mg^{2+} added, which were made at 23.5 ± 0.2°C, all measurements were made at 24.9 ± 0.2°C.

2.2. Materials and sample preparations

The disodium salts of the nucleotides AMP (manufacturer's no. A1752), CMP (C1006), UMP (6375), purine (P6880), pyrimidine (P0131), cytidine (C9505), uridine (U3750), 2′,3′-*O*-isopropylidenecytidine hydrochloride (I3003) and 2′,3′-*O*-isopropylideneuridine (I5127) were from Sigma Chemical Co. Theophylline-7-acetic acid was purchased from Fluka AG, Buchs, Switzerland. Water-free cadmium chloride and sulphate salts of magnesium and zinc (both with $7H_2O$) were obtained from Merck AG, Darmstadt, F.R.G. The solvent (^2H_2O) was purchased from Norsk Hydro, Rjukan, Norway.

The nucleotides, nucleosides, 'nucleobases' and isopropyl nucleosides were used without further purification. Solutions were made as molal concentrations by weight, directly in the thin-wall 5-mm NMR tubes. The sulphate salts of magnesium and zinc were dried at 200 and 280°C, respectively, for sevral hours to remove water. The pH values of the solutions were in the range 5–8. 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.

CMP and UMP with Mg^{2+} added up to 0.4 molal ([CMP] = [Mg^{2+}] = 0.4 molal and [UMP] = [Mg^{2+}] = 0.4 molal) and AMP with Mg^{2+} added ([AMP] = Mg^{2+}]) up to the solubility limit in ^2H_2O, were studied and fitted to the two models described. The system [GMP] = [Mg^{2+}] could not be studied due to precipitation already at low concentration. Precipitation also occurred at low concentrations for all four nucleotides with Zn^{2+} or Cd^{2+} added, and these systems were therefore amenable for study. The nucleosides, cytidine and uridine, could be studied with either of these three metal ions and purine was studied with Mg^{2+}. Purine with Zn^{2+} or Cd^{2+} added precipitated already at low concentrations in water.

3. Measurement and calculational approach

The present technique of studying molecular association is particularly suitable and straightforward for the investigation of the binding of small substrates to large macromolecules. This is seen from the expression for the time-averaged self-diffusion coefficient in a multisite situation:

$$D_{obs} = \sum_i p_i D_i \qquad (2)$$

The D_i are closely dependent on aggregate size, since the fraction of reach species is weighted according to its diffusion coefficient, which decreases in a monotonic fashion with aggregate size. The partial binding of a small molecule to a macromolecule or supramolecular species will manifest itself in a rather receptive manner in its time-averaged self-diffusion coefficients (typically a 2 decade ratio). In applying the self-diffusion technique for the investigation of aggregation phenomena of the present kind, one notes that the method necessarily will be biased to contributions from monomers and lower oligomers.

The binding constants involved in nucleotide and nucleoside association are known to be rather small, making it necessary to monitor a relatively large concentration span in order to induce significant changes in observed diffusion coefficients. A complication arising from the increase in total concentration is the assessment of obstruction effects; the diffusional path of a particular species will increase due to the presence of aggregate and leads to a decrease of diffusion coefficient.

The calculations and the computer simulations on observed diffusion data were made as previously described [37]. In brief outline the observed diffusion coefficient, D_{obs}, in a system of associating molecules subject to the condition of rapid exchange is assumed to given by eq. 2. By combining eq. 2 with the appropriate assumed association model (providing the p values as a function of concentration), and providing appropriate corrections for obstruction [52,53] and for hydrodynamic friction [54,55], the experimental data can be fitted to different sets of association parameters through computer simulation.

A series of iterative non-linear least-square computer programs (minimizing the sum over all $(D_{obs} - D_{calc})^2$ under the constraints of the aggregation model and eq. 2) were developed for the simulations of self-diffusion data within the different aggregation models. Details of algorithms and computational procedures will be published elsewhere [56].

Two models were considered (see section 1):

(1) Model 1, indefinite aggregation with all steps equal was simulated with the ASGE11 computer program [37,56].

(2) Model 2, indefinite aggregation, dimerization step unique, i.e.:

$$\begin{aligned} A + A &\rightleftarrows A_2 \quad K_1 = [A_2]/[A][A] \\ A_2 + A &\rightleftarrows A_3 \quad K_2 = [A_3]/[A_2][A] \\ A_i + A &\rightleftarrows A_{i+1} \quad K_i = [A_{i+1}]/[A_i][A] \end{aligned} \qquad (3)$$

where 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.

As in our previous study, numerous additional simulations were made with different values of the numerical values of the obstruction correction factors. It was confirmed that the general conclusions of the present paper are insensitive to and the numerical values of the fitted equilibrium con-

stants are only moderately sensitive to any physically reasonable span of obstruction correction factors.

4. Results and discussion

4.1. Observed diffusion data and their analysis

Tables 1–5 summarize the observed diffusion coefficients.

Table 1

Purine self-diffusion coefficients of purine and Mg(purine)$^{2+}$ in ^2H_2O

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

Purine		Purine + Mg^{2+}	
C molal)	D_{obs}	C (molal)	D_{obs}
0.0317	0.716	0.0346	0.712
0.0512	0.708	0.0512	0.666
0.0663	0.714	0.0650	0.657
0.0860	0.691	0.0836	0.676
0.112	0.668	0.101	0.636
0.132	0.668	0.117	0.636
0.150	0.657	0.132	0.601
0.178	0.639	0.151	0.582
0.205	0.636	0.174	0.549
0.228	0.616	0.198	0.537
0.251	0.615	0.226	0.518
0.303	0.598	0.251	0.490
0.354	0.575		

In previous work [37] we have demonstrated that the monomer-single n-mer model is generally inconsistent with observed diffusion data on mononucleotide systems; therefore this model has not been considered in the present data analyses.

In Models 1 and 2 self-diffusion coefficients calculated from hydrodynamics are used in the simulations. These are physically reasonable a priori; it is the aggregation model itself that is subject to fitting procedures. For the uncomplexed purine, cytidine, uridine and the isopropyl nucleotides, both models fit the experimental data almost equally well. Model 2 provides a significantly better agreement between experiment and simulation than Model 1, for all the metal ion complexes, supporting the general conclusions of our previous paper [37].

4.2. Association of purine, cytidine and uridine

The two models fit the data almost equally well for these three compounds (see table 6). In contrast to the nucleotides studied earlier [37], the same large difference does not occur between the first (dimerization) constant and the aggregation constant for the higher steps, when regarding Model 2. Numerical values from the literature [1,26] show that purine associates to almost the same degree as AMP, and that cytidine and uridine

Table 2

Cytidine self-diffusion coefficients in cytidine and Met(cytidine)$^{2+}$ ^2H_2O systems

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

Cytidine		Cytidine + Mg^{2+}		Cytidine + Zn^{2+}		Cytidine + Cd^{2+}	
C (molal)	D_{obs}	C (molal)	D_{obs}	C (molal)	D_{obs}	C (molal)	D_{obs}
0.0219	0.486	0.0352	0.473	0.0284	0.476	0.0337	0.472
0.0340	0.478	0.0513	0.458	0.0488	0.454	0.0483	0.460
0.0537	0.465	0.0739	0.438	0.0953	0.423	0.0674	0.425
0.0684	0.468	0.0849	0.440	0.122	0.410	0.0935	0.408
0.106	0.460	0.107	0.433	0.148	0.385	0.129	0.372
0.126	0.450	0.125	0.411	0.169	0.380	0.146	0.374
0.149	0.434	0.148	0.401	0.192	0.343	0.206	0.337
0.175	0.421	0.176	0.383	0.244	0.315	0.225	0.347
0.200	0.426	0.193	0.374	0.283	0.304	0.250	0.317
0.254	0.411	0.221	0.367	0.340	0.272	0.279	0.310
0.304	0.396	0.251	0.346	0.400	0.249	0.333	0.290
0.352	0.377					0.377	0.272
0.404	0.369						

Table 3

Uridine self-diffusion coefficients in uridine and Met(uridine)$^{2+}$ ^2H_2O systems

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

Uridine		Uridine + Mg^{2+}		Uridine + Zn^{2+}		Uridine + Cd^{2+}	
C (molal)	D_{obs}	C (molal)	D_{obs}	C (molal)	D_{obs}	C (molal)	D_{obs}
0.0090	0.495	0.0295	0.477	0.0356	0.486	0.0333	0.498
0.0178	0.503	0.0485	0.467	0.0612	0.464	0.0529	0.497
0.0383	0.493	0.0585	0.476	0.0738	0.471	0.106	0.446
0.0513	0.492	0.0799	0.443	0.0972	0.441	0.123	0.458
0.0804	0.456	0.0957	0.442	0.121	0.447	0.150	0.436
0.103	0.472	0.123	0.430	0.147	0.420	0.179	0.407
0.127	0.470	0.146	0.417	0.173	0.409	0.200	0.406
0.152	0.452	0.173	0.401	0.189	0.386	0.226	0.406
0.172	0.457	0.204	0.388	0.245	0.356	0.245	0.399
0.199	0.439	0.215	0.395	0.313	0.328	0.306	0.383
0.247	0.422	0.245	0.372	0.337	0.320	0.346	0.365
0.305	0.434			0.386	0.300	0.410	0.347
0.354	0.399						
0.397	0.403						

aggregation is characterized by lower association constants. When considering the total aggregation, regarding both K_1 and K_2, the degree of association of purine though seems comparable to the association of AMP, where the two evaluated association constants are $K_1 = 0.324$ M^{-1} and $K_2 = 9.49$ M^{-1} [37]. It is generally accepted from other measurements and methods (see, for example, ref. 36) that the purine derivatives have a greater tendency to associate than pyrimidine derivatives, due to the greater ring system in purines. As based on Model 2, this tendency also holds for purine and the two pyrimidine nucleosides, cytidine and uridine, in the present investigation, though the values obtained for the self-aggregation of purine are only slightly larger than the corresponding values for cytidine.

Model 1, with all equilibrium constants equal,

Table 4

Nucleotide self-diffusion coefficients in nucleotide Mg^{2+} ^2H_2O systems

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

AMP + Mg^{2+}		CMP + Mg^{2+}		UMP + Mg^{2+}	
C (molal)	D_{obs}	C (molal)	D_{obs}	C (molal)	D_{obs}
0.0357	0.358	0.0357	0.336	0.0338	0.370
0.0486	0.333	0.0506	0.325	0.0524	0.338
0.0707	0.305	0.0722	0.317	0.0738	0.304
0.101	0.282	0.104	0.283	0.105	0.282
0.125	0.251	0.125	0.278	0.121	0.300
0.155	0.236	0.151	0.244	0.148	0.270
0.182	0.211	0.202	0.232	0.196	0.227
0.197	0.207	0.259	0.200	0.245	0.213
0.231	0.188	0.276	0.181	0.286	0.195
0.246	0.180	0.385	0.144	0.355	0.171
0.277	0.175				

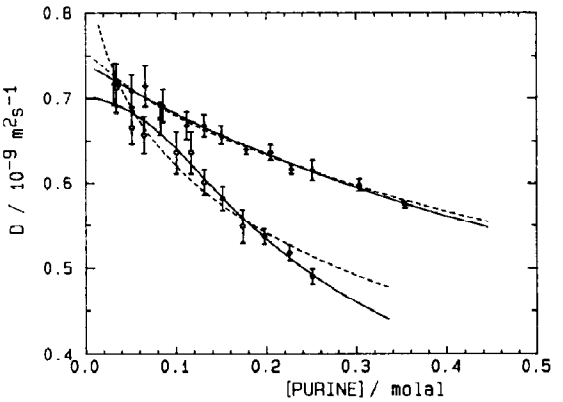

Fig. 1. The concentration dependence of the purine self-diffusion coefficient in purine/water (◆) and Mg^{2+}/purine/water systems (○). Curves are calculated from the fitted parameters for the association Models 1 (----) and 2 (———).

Table 5

Self-diffusion coefficients of isopropyl nucleosides, theophylline-7-acetic acid and pyrimidine in ^2H_2O

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

Isopropylcytidine		Isopropyluridine		Theophylline-7-acetic acid		Pyrimidine	
C (molal)	D_{obs}	C (molal)	D_{obs}	C (molal)	D_{obs}	C (molal)	D_{obs}
0.0095	0.467	0.0100	0.471	0.0372	0.472	0.0337	0.938
0.0182	0.451	0.0189	0.455	0.0514	0.477	0.0516	0.946
0.0300	0.448	0.0270	0.454	0.0645	0.475	0.0704	0.930
0.0485	0.443	0.0305	0.445	0.0814	0.459	0.101	0.921
0.0623	0.436	0.0401	0.450	0.102	0.452	0.118	0.922
0.0747	0.434	0.0509	0.434	0.116	0.431	0.151	0.930
0.0848	0.431	0.0550	0.438	0.128	0.436	0.175	0.931
0.103	0.424	0.0616	0.430	0.201	0.413	0.218	0.927
0.113	0.419	0.0650	0.434	0.252	0.400	0.256	0.907
0.130	0.420	0.0709	0.430	0.300	0.380	0.294	0.914
0.139	0.408	0.0808	0.433	0.355	0.361	0.346	0.900
0.146	0.411	0.0947	0.426	0.356	0.361	0.412	0.885
0.163	0.398	0.1031	0.421	0.392	0.346		
0.168	0.399			0.432	0.335		

reproduces the experimental data for uridine, but does not reproduce those for purine and cytidine as well as Model 2.

4.3. *Influence of divalent metal ions on the self-association of purine and nucleosides*

Comparision of the association constants for the nucleoside-metal ion complexes with the corresponding values for uncomplexed nucleosides clearly shows that addition of divalent metal ions to the nucleoside solutions favours self-aggregation. For both cytidine and uridine the higher association constant (K_2) is increased by a factor of 2–3 upon addition of Mg^{2+} while the first (dimerization) constant stays at the same level. A similar increase of the higher association constant is observed when Zn^{2+} is added to the nucleosides.

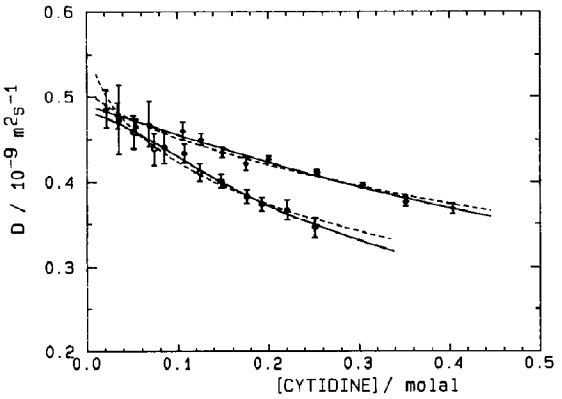

Fig. 2. The concentration dependence of the cytidine self-diffusion coefficient in cytidine/water (◆) and Mg^{2+}/cytidine/water systems (○). Curves are calculated from the fitted parameters for the association Models 1 (----) and 2 (———).

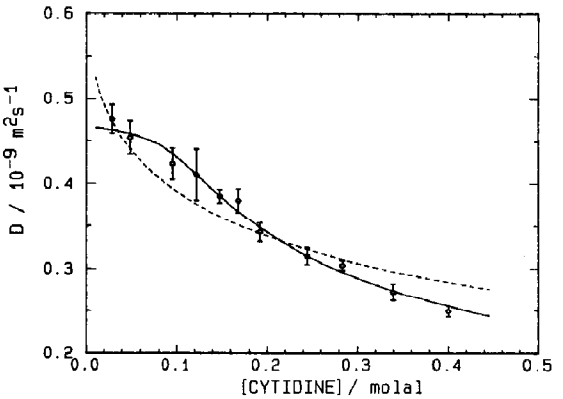

Fig. 3. The concentration dependence of the cytidine self-diffusion coefficient in the Zn^{2+}/cytidine/water system. Curves are calculated from the fitted parameters for the association Models 1 (----) and 2 (———).

Table 6

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

K_1, K_2 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.

Cooperative indefinite aggregation				Indefinite aggregation		
K_1 ($molal^{-1}$)	K_2 ($molal^{-1}$)	D_1	FOBJ	K ($molal^{-1}$)	D_1	FOBJ
Purine						
2.03	3.66	0.740	0.00082	4.37	0.757	0.00102
Cytidine						
1.42	3.68	0.490	0.00152	4.83	0.506	0.00247
Uridine						
1.96	2.50	0.504	0.00547	2.48	0.507	0.00552
Purine + Mg^{2+}						
0.496	7.52	0.703	0.00396	47.5	0.889	0.01113
Cytidine + Mg^{2+}						
0.145	7.21	0.453	0.00290	28.6	0.563	0.00285
Cytidine + Zn^{2+}						
0.219	7.99	0.467	0.00431	67.7	0.592	0.05129
Cytidine + Cd^{2+}						
2.99	13.4	0.497	0.00518	69.3	0.598	0.01085
Uridine + Mg^{2+}						
2.09	6.79	0.493	0.00172	14.0	0.534	0.00257
Uridine + Zn^{2+}						
0.450	6.58	0.487	0.00240	53.5	0.632	0.01841
Uridine + Cd^{2+}						
4.27	8.20	0.529	0.00333	15.5	0.564	0.00365
AMP + Mg^{2+}						
0.120	13.9	0.349	0.00671	84.1	0.393	0.14034
UMP + Mg^{2+}						
0.34	11.7	0.356	0.02044	69.9	0.415	0.10522
CMP + Mg^{2+}						
0.008	10.8	0.327	0.02112	67.5	0.388	0.18592
Isopropylcytidine						
1.96	4.37	0.463	0.00096	4.02	0.469	0.00109
Isopropyluridine						
42.7	6.86	0.499	0.00076	4.72	0.471	0.00120
Theophylline-7-acetic acid						
1.11	4.67	0.487	0.00257	12.4	0.540	0.00490
Pyrimidine						
0.00	0.00	0.956	0.00149	0.00	0.956	0.00149

Here the first constant, K_1, becomes very low. When dimerization has occurred, the aggregation seems to proceed in the same order with Zn^{2+} added as with Mg^{2+}. Cd^{2+} addition to the nucleoside solutions increases both the association constants, the first by a factor of 2–3 and the higher one by a factor of 3–4. The self-aggregation of the pyrimidine nucleosides, cytidine and uridine, upon metal ion addition, seems to increase in the following series: $Zn^{2+} \leqslant Mg^{2+} < Cd^{2+}$. Sigel et al. [26] have found that the self-aggregation of both $Zn(ATP)^{2-}$ and $Cd(ATP)^{2-}$ is significantly larger than for $Mg(ATP)^{2-}$, but the value of the association constant for $Zn(ITP)^{2-}$ is similar or only slight larger than the value for $Mg(ITP)^{2-}$. Due to precipitation upon addition of divalent metal ions, there are only a limited number of complexes with nucleotides and nucleosides that have been, and can be, studied.

Addition of Mg^{2+} to purine solutions leads to

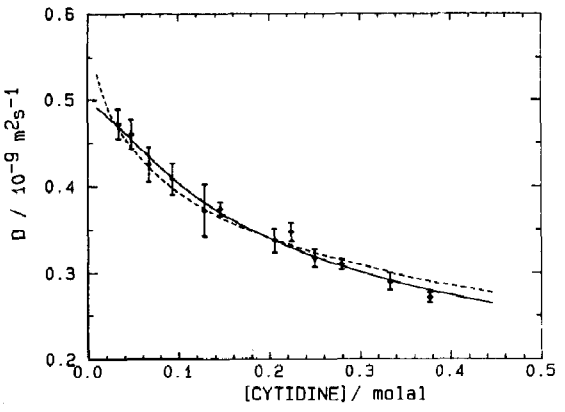

Fig. 4. The concentration dependence of the cytidine self-diffusion coefficient in the Cd^{2+}/cytidine/water system. Curves are calculated from the fitted parameters for the association Models 1 (----) and 2 (———).

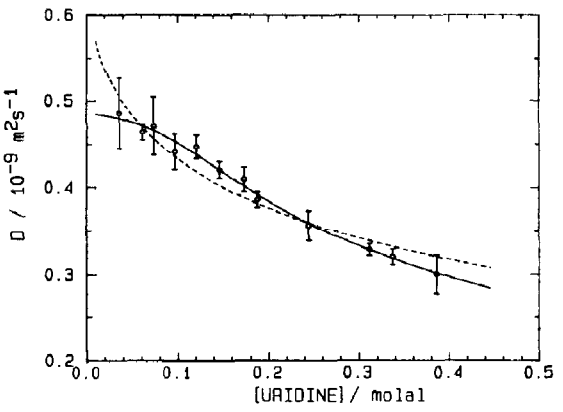

Fig. 6. The concentration dependence of the uridine self-diffusion coefficient in the Zn^{2+}/uridine/water system. Curves are calculated from the fitted parameters for the association Models 1 (----) and 2 (———).

aggregation that is characterized by a lower first association constant and a larger constant for higher aggregation steps compared to purine. The total aggregation upon Mg^{2+} addition therefore does not seem to increase as much for purine as for cytidine and uridine.

Aggregation constants for such nucleoside-metal ion complexes have not been found in the literature, with the exception of a study by Sigel et al. [26] who by chemical shift NMR studies found that a Cd(cytidine)$^{2+}$ complex is formed. Model 1 poorly reproduces the experimental data for these metal ion complexes; Model 2 gives a considerably better agreement with experimental observations, however.

4.4. Influence of divalent metal ions on the self-association of nucleotides

Comparison of the association constants in the Mg^{2+}/XMP systems (X = A, C or U) with the constants for uncomplexed XMP^{2-} shows that the

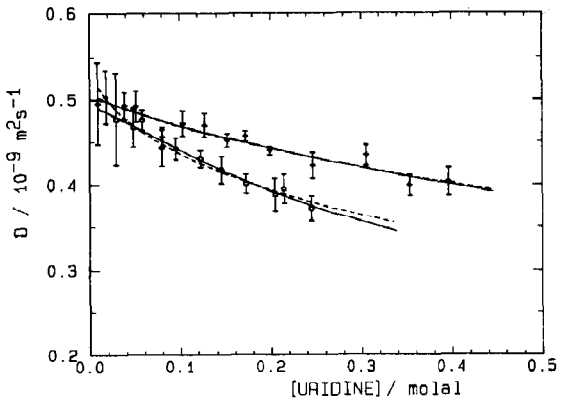

Fig. 5. The concentration dependence of the uridine self-diffusion coefficient in uridine/water (◆) and Mg^{2+}/uridine/water systems (○). Curves are calculated from the fitted parameters for the association Models 1 (----) and 2 (———).

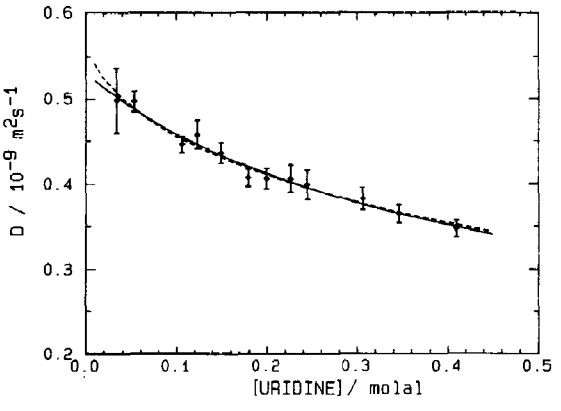

Fig. 7. The concentration dependence of the uridine self-diffusion coefficient in the Cd^{2+}/uridine/water system. Curves are calculated from the fitted parameters for the association Models 1 (----) and 2 (———).

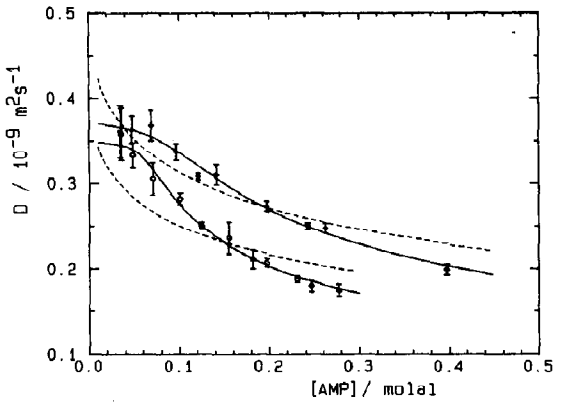

Fig. 8. The concentration dependence of the AMP self-diffusion coefficient in the Mg^{2+}/AMP^{2-}/water system (○) (and AMP^{2-}/water (◆) at the same temperature). Curves are calculated from the fitted parameters for the association Models 1 (----) and 2 (———).

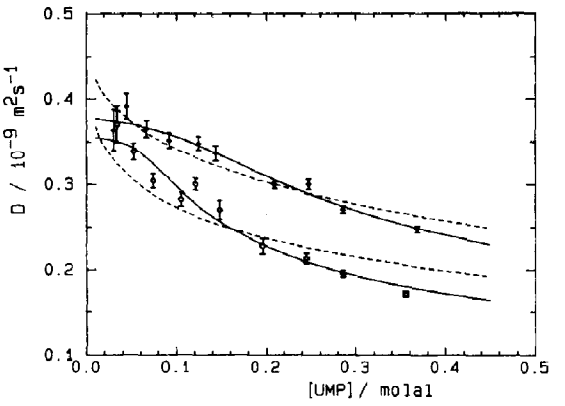

Fig. 10. The concentration dependence of the UMP self-diffusion coefficient in the Mg^{2+}/UMP^{2-}/water system (○) (and UMP^{2-}/water (◆) at the same temperature). Curves are calculated from the fitted parameters for the association Models 1 (----) and 2 (———).

self-aggregation tendency is promoted by a factor of about 2 for the second association constant in Model 2, upon Mg^{2+} addition. The first association constant remains low and about the same as for uncomplexed XMP^{2-}. The tendency with greater association constants for AMP than for the pyrimidine nucleotides, CMP and UMP [37], is seen even for the nucleotide-metal ion complexes in the present investigation. However, the increase in association constants is about the same for the three nucleotides, when Mg^{2+} is added. Similar results have been observed previously; Sigel et al. [26] have, for example, found that K increases by a factor of about 3 when Mg^{2+} is added to ATP solutions.

The first model, with all aggregation constants equal, poorly reproduces the experimental data for the nucleotide-metal ion complexes.

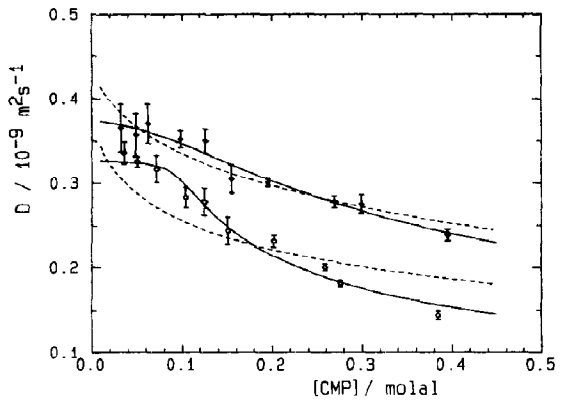

Fig. 9. The concentration dependence of the CMP self-diffusion coefficient in the Mg^{2+}/CMP^{2-}/water system (○) (and CMP^{2-}/water (◆) at the same temperature). Curves are calculated from the fitted parameters for the association Models 1 (----) and 2 (———).

4.5. Association of isopropyl nucleosides, pyrimidine and theophylline-7-acetic acid

Due to the limited solubility of the isopropyl nucleosides, especially isopropyluridine, it is difficult to compare these results with the results obtained for purine, nucleosides, nucleotides and their metal ion complexes. The application of Model 2 results in a very high first (dimerization) constant for isopropyluridine, and an association constant for the higher steps which is a magnitude lower. This is not physically reasonable if the aggregation is due primarily to dispersive interactions [35,57]. Regarding Model 1, one finds that the association constant for these two compounds is of the same order as for cytidine and uridine, as expected. Methylation often increases the aggregation (for example, caffeine compared to purine),

but methylation at the pentose unit has been shown to have little effect [36]. If the aggregation is primarily due to stacking of the planar bases, it is not reasonable to believe that an isopropyl group in the 2',3' position of the pentose should influence the aggregation.

Pyrimidine diffusion data did not reveal any aggregation; all aggregation constants became zero in both models studies.

Theophylline-7-acetic acid has two methyl groups on the purine ring system and is a compound well suited for FT-PGSE studies because of the favourable spin relaxation characteristics of its methyl signals. The values obtained by Model 2 for this compound are of the same order as those observed for the nucleosides and purine, but with a greater difference in the two association constants, K_1 and K_2.

5. Conclusions

With regard to a comparison with previous data on similar systems in the literature one must point out that equilibrium constants for different aggregation models are, of course, not directly intercomparable. In general, this self-diffusion approach gives somewhat higher numerical values than previously published values for the same aggregation model. Another reason for an apparent discrepancy is that, unlike activity-based data (such as those obtained from osmometry and vapour pressure measurements), the self-diffusion approach monitors concentration-related quantities, with no direct influence from non-ideal interactions in the thermodynamic sense.

A cooperative aggregation model leads to better agreement with experimental results [3,57,58], as compared to the simple isodesmic model. For all the compounds studied in the present investigation, it is also shown that Model 2 agrees better with the experimental results than Model 1. At the present stage we did not find it justifiable to continue the search for better agreement between model and experiment because the meaningful limit had been reached (considering the precision of the data and the fits between model and experiment).

In conclusion, one may state that divalent metal ions such as Mg^{2+}, Zn^{2+} and Cd^{2+} clearly promote self-aggregation of both nucleotides and nucleosides. The tendency is greater for Cd^{2+} added to the nucleosides than Mg^{2+} and Zn^{2+}.

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