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STRUCTURE–RETENTION RELATIONSHIPS: CHROMATOGRAPHIC BEHAVIOUR AND PROPERTIES OF NUCLEOTIDE–CATION COMPLEXES

AHARON S. COHEN and ELI GRUSHKA*
Department of Inorganic and Analytical Chemistry, The Hebrew University of Jerusalem, Jerusalem (Israel)
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SUMMARY

Nucleotides, nucleosides and their bases are known to form complexes with metal cations, some of which are essential for the proper functioning of these compounds. The cations have several possible roles, all of which cause some changes in the parent molecule's spacial arrangement and/or charge. Hence chromatographic retention, being sensitive to these two parameters, should differ for complexed and uncomplexed nucleotides. The retentions were obtained using mobile phases containing various concentrations of one of four metal cations, Mg(II), Ni(II), Zn(II) and Cu(II). These were then compared with values obtained without the cations. As expected, the nature and concentration of the metal ion in the mobile phase affected the chromatographic behaviour. In all instances Mg(II) caused a decrease in the retention times, as measured from the capacity ratios (k'). The presence of Ni(II) or Zn(II) in the mobile phase caused an initial decrease and then, as the concentration of these metal ions increased further, k' increased. The nucleotide retentions increased when Cu(II) was added to the mobile phase. The possible formation of an intramolecular macrochelate can be related to the pattern of the retention times. In addition, it is shown that the capacity ratios can be used to predict properties such as base acidity and dephosphorylation.

INTRODUCTION

At least ten different metal cations are known to be essential to higher forms of life. In addition to potassium and sodium, the most abundant elements in the biosphere are iron, zinc, magnesium, calcium and copper. Nickel, vanadium, chromium and manganese are also now recognized as essential trace elements[1]. One of the major roles played by the metal cations is in metalloenzymes. The cations are essential to enzymatic activity, as they mediate in the enzyme–substrate interactions[2–4]. In addition, both enzyme and substrate can complex metal cations strongly.

The ternary nucleotide–metal cation–amino acid complex can serve as the simplest possible model of an enzymatic active centre. Our long-range aim is to characterize such ternary complexes chromatographically. However, before this task can be accomplished, the behaviour of the binary nucleotide–cation, amino acid–cation

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and nucleotide–amino acid complexes must be ascertained. This paper deals with the retention behaviour of nucleotide–metal cation complexes.

Metal interactions with nucleotides, nucleosides and their bases have been widely reported. However, their exact function is not always known. Their effects on the behaviour of the above compounds have been documented by many workers. For example, Eichhorn and his group[5–7] have reported that Ca(II) and Mg(II) enhance the stability of DNA, whereas Cu(II) acts in the opposite direction. This was surmised from an increase or a decrease in the melting point of DNA. Their explanation was simple[7]: metal ions that coordinate only to the phosphates stabilize the macromolecule. On the other hand, metal ions that may interact with the base cause destabilization. This explanation is in line with current assumptions that cations can interact with several functional groups of nucleotides.

Sigel and Amsler[8] indicated that the possible roles of the metal cations in the complex are (1) charge neutralization; (2) polarization; (3) strain induction; (4) template formation; (5) coordination to leaving groups; and (6) tight coordination to the transition state. Implicit in the above list are items such as steric deformation, geometric rearrangement and hydrophobicity. Techniques such as T-jump[9,10], NMR[11–16], UV spectroscopy[17], Raman spectroscopy[16] and optical rotatory dispersion[18] have been used to investigate these roles and structural aspects of the complexes. As the above-mentioned roles basically mean changes in the charge and/or spatial configuration of the complex compared with the organic moiety alone, it is clear that reversed-phase liquid chromatography can serve as a tool in studying the nature of these complexes.

Horváth *et al.*[19] and Grushka and Chow[20] showed that liquid chromatography can be used to measure the formation constants of metal–nucleotide complexes. Brown and co-workers[21,22] recently studied the chromatographic retention behaviour of nucleotides, nucleosides and their bases, and have compared it with parameters such as stacking and ionization. In this paper we shall discuss the retention of metal–nucleotide complexes and factors that affect it. It will be shown that correlations exist between retention and other physico-chemical properties of the complexes.

EXPERIMENTAL

Materials

All nucleoitides, nucleosides and their bases were purchased from Sigma (Sigma Israel, Tel-Aviv, Israel). The metal sulphate salts were obtained from Mallinckrodt (St. Louis, MO, U.S.A.).

The mobile phase buffer was prepared using 18 MΩ water prepared in our laboratory.

Instrumentation

The chromatographic studies were carried out using a Spectra-Physics Model 8000 liquid chromatograph with a data system and a variable-wavelength UV–visible detector. The column (100 × 4.5 mm I.D.) was packed with 3-μm reversed-phase (HS-3 C_{18}) particles (Perkin-Elmer, Norwalk, CT, U.S.A.).

Procedure

The mobile phase was an aqueous acetate buffer of pH 5.6 ± 0.1 and 0.04 *M* ionic strength. The following cation concentration ranges were prepared in this buffer: [Mg] = $5 \cdot 10^{-4}$–$6 \cdot 10^{-3}$ *M*; [Ni] = $5 \cdot 10^{-5}$–$5 \cdot 10^{-3}$ *M*; [Zn] = $5 \cdot 10^{-4}$–$6 \cdot 10^{-3}$ *M*; and [Cu] = $5 \cdot 10^{-4}$–$3 \cdot 10^{-3}$ *M*. The concentrations of the nucleotides, nucleosides and bases were 10^{-3} *M* and 10 μl of these solutes were injected. The mobile phase flow-rate was kept constant at 1.0 ml/min and the column temperature was constant at 37°C.

RESULTS AND DISCUSSION

Retention of bases and nucleosides

As indicated in the Introduction, chromatography can be used to obtain the formation constants of metal–nucleotide complexes[19,20]. However, the aim of this work was to correlate the retention with physico-chemical properties of the complexes, and not to measure the formation constants. The concentration of the solutes was not in the correct range for such measurements[19]. It should be pointed out, perhaps, that the capacity factor of the nucleotide was a function of their concentration. This hitherto unreported concentration dependence is now a subject of a separate study.

The solutes studied were the following: adenine, adenosine [studied in the presence of Cu(II) only], AMP, ADP, ATP, cAMP, guanosine, GMP, GDP, GTP, cytosine, cytidine, CMP, CDP, CTP, cCMP, uracil, uridine, UMP, UDP and UTP. The capacity ratios of all these solutes, in the presence of metal ions, are given in Table I. Table II gives the capacity factor of some nucleosides, bases and two cyclic nucleotide monophosphates (cNMP). It can be seen that the effect of the metal ion is mostly negligible; on the whole, the capacity ratios seem to decrease slightly in the presence of the cations. This is perhaps due to ionic strength effects, as the concentration of the metal cations was 50 times less than that of the acetate buffer alone. The obvious exception is adenine in the presence of Cu ions. It will be shown shortly that Cu ions affect the retention behaviour of nucleotides in a different manner than the other metals studied. Here, it is sufficient to point out that at pH 5.6 copper is able to ionize adenine and form a complex. The affinity of all other solutes in Table II, at pH 5.6, towards metal ions is very small. Examination of the complete set of data shows that, with the exception of adenine, the k' values of all the solutes mentioned in Table II were to a large extent independent of the metal ion concentration. The arguments advanced by Brown and Grushka[21] can be used to correlate these results: adenine is known to form stacks easily, much more so than the other solutes in Table II. Moreover, metal ions are thought to enhance stacking, particularly copper. Hence factors that affect stacking also seem to influence the retention. The retention order which, in general, decreases in the series Cu > Zn > Ni > Mg, can also be explained by the above discussion.

Retention of nucleotides

The effect of metal ions is much more noticeable in the case of nucleotides. For example, Fig. 1 shows the change in the k' values of AMP, ADP and ATP as a function of Mg(II) concentration. In this work, the retention order for nucleotides

TABLE I

DEPENDENCE OF k' VALUES OF THE SOLUTES STUDIED ON THE TYPE AND CONCENTRATION OF THE METAL IONS

Solute	k'_0 *(pH = 5.6)*	*Mg(II) concentration (M)*				
		$5 \cdot 10^{-4}$	$10 \cdot 10^{-4}$	$20 \cdot 10^{-4}$	$40 \cdot 10^{-4}$	$60 \cdot 10^{-4}$
Adenine	5.266	4.960	4.899	4.902	4.902	4.990
Adenosine						
AMP	2.312	2.200	2.050	1.920	1.920	1.890
cAMP	9.650	10.080	10.000	10.064	10.064	10.000
ADP	2.846	1.476	0.992	0.899	0.899	0.829
ATP	2.990	1.382	0.823	0.743	0.743	0.658
Guanosine	6.786	6.133	6.038	6.210	6.210	5.658
GMP	0.680	0.652	0.584	0.534	0.533	0.553
GDP	0.739	0.377	0.245	0.239	0.239	0.223
GTP	1.671	0.332	0.200	0.180	0.180	0.171
Cytosine	0.547	0.507	0.510	0.510	0.510	0.487
Cytidine	1.387	1.280	1.216	1.184	1.840	1.263
CMP	0.213	0.168	0.121	0.109	0.109	0.105
cCMP	2.490	2.493	2.500	2.499	2.500	2.500
CDP	0.320	0.065	0.052	0.050	0.050	0.040
CTP	1.320	0.044	0.030	0.026	0.026	0.026
Uracil	0.795	0.795	0.798	0.798	0.798	0.802
Uridine	2.176	1.922	1.880	1.836	1.830	1.778
UMP	0.270	0.252	0.256	0.250	0.250	0.239
UDP	0.541	0.100	0.099	0.079	0.079	0.078
UTP	0.813	0.071	0.061	0.051	0.051	0.052

Solute	k'_0 *(pH = 5.6)*	*Ni(II) concentration (M)*						
		$5 \cdot 10^{-4}$	$10 \cdot 10^{-4}$	$20 \cdot 10^{-4}$	$25 \cdot 10^{-4}$	$30 \cdot 10^{-4}$	$50 \cdot 10^{-4}$	$5 \cdot 10^{-5}$
Adenine	5.266	4.723	4.733	4.797	4.800	4.827	4.815	
Adenosine								
AMP	2.312	1.908	1.933	1.960	1.960	1.973	1.987	2.116
cAMP	9.650	8.881	8.990	8.986	8.986	9.013	9.000	
ADP	2.846	0.921	1.120	1.200	1.213	1.227	1.236	2.460
ATP	2.990	0.644	0.747	0.919	0.920	0.920	0.947	2.840
Guanosine	6.786	5.855	5.600	5.905	5.986	6.000	5.987	
GMP	0.680	0.513	0.512	0.527	0.534	0.534	0.539	0.636
GDP	0.739	0.329	0.413	0.459	0.466	0.480	0.473	0.701
GTP	1.671	0.224	0.347	0.405	0.413	0.426	0.447	0.818
Cytosine	0.547	0.513	0.507		0.507		0.513	
Cytidine	1.387	1.276	1.280		1.276		1.276	
CMP	0.213	0.158	0.202	0.202	0.200	0.200	0.197	
cCMP	2.490	2.500	2.467		2.453	2.453	2.421	
CDP	0.320	0.145	0.173	0.187	0.187	0.200	0.197	
CTP	1.320	0.066	0.107	0.135	0.147	0.147	0.158	
Uracil	0.795	0.723	0.720		0.793	0.734	0.723	
Uridine	2.176	1.895	1.880		1.907	1.907	1.895	
UMP	0.270	0.263	0.253	0.297	0.293	0.306	0.303	
UDP	0.541	0.237	0.280	0.297	0.293	0.306	0.303	
UTP	0.813	0.132	0.240	0.250	0.280	0.293	0.302	

Zn(II) concentration (M)							
$5 \cdot 10^{-4}$	$10 \cdot 10^{-4}$	$15 \cdot 10^{-4}$	$20 \cdot 10^{-4}$	$25 \cdot 10^{-4}$	$30 \cdot 10^{-4}$	$50 \cdot 10^{-4}$	$80 \cdot 10^{-4}$
4.987	4.987	4.987	4.908	5.040		4.987	4.987
2.186	2.227	2.253	2.263	2.267	2.284	2.293	2.000
8.826	9.027		9.460	9.680		8.773	9.600
1.920	2.067	2.120	2.145	2.160	2.189	2.200	2.107
1.386	1.680	1.787	1.816	1.867	1.919	1.947	1.800
6.067	6.093	6.107	6.118	6.093	6.190	6.133	5.973
0.640	0.720	0.733	0.724	0.746	0.743	0.747	0.733
0.613	0.680	0.707	0.711	0.720	0.729	0.733	0.706
0.440	0.587	0.640	0.645	0.667	0.689	0.707	0.640
0.520	0.520	0.520	0.526	0.520		0.507	0.546
1.280	1.281	1.280	1.276	1.280		1.267	1.280
0.173	0.187	0.187	0.184	0.187	0.189	0.200	0.173
2.506	2.480		2.500		2.500	2.467	2.107
0.133	0.160	0.173	0.171	0.173	0.176	0.187	0.160
0.093	0.147	0.160	0.158	0.173	0.176	0.187	0.147
0.800	0.800	0.800	0.803	0.813	0.797	0.813	0.747
2.013	2.013	2.000	2.013	2.014	2.013	2.013	1.947
0.267	0.280	0.280	0.276	0.280	0.284	0.280	0.267
0.187	0.227	0.227	0.237	0.240	0.243	0.253	0.226
0.107	0.173	0.187	0.211	0.227	0.216	0.227	0.200

		Cu(II) concentration (M)					
$20 \cdot 10^{-5}$	$40 \cdot 10^{-5}$	$5 \cdot 10^{-4}$	$10 \cdot 10^{-4}$	$15 \cdot 10^{-4}$	$20 \cdot 10^{-4}$	$25 \cdot 10^{-4}$	$30 \cdot 10^{-4}$
		10.432	21.243	22.851			
		17.910	18.391	18.420			18.520
1.921	2.000	2.171	2.432	2.486	2.706	2.800	2.906
		9.526					
1.816	0.880	4.526	5.648	5.797	6.106	6.146	6.200
1.550	0.600	5.026	5.889	6.406	6.506	6.600	6.710
		6.416	6.473	6.234	6.380		6.427
0.579	0.507	1.158	1.486	1.486	1.540	1.573	1.607
0.447	0.280	1.421	1.787	1.891	1.950	1.920	2.027
0.397	0.160	2.434	2.726	2.851	2.900	2.933	2.973
		0.605	0.600	0.608	0.613	0.613	0.613
		1.355	1.386	1.378	1.380	1.400	1.413
		0.473	0.513	0.514	0.530	0.526	0.530
		0.618	0.893	0.946	0.990	1.013	10.26
		0.895	1.120	1.202	1.220	1.223	1.230
		0.813			0.803	0.802	0.813
		2.013	2.000		2.013	1.987	2.080
		0.373	0.384	0.405	0.400	0.400	0.426
		0.786	0.922	0.959	1.066	1.013	1.040
		1.066	1.169	1.216	1.240	1.250	1.230

TABLE II

EFFECT OF M^{2+} ON k' VALUES OF SEVERAL SOLUTES

[M] = $2 \cdot 10^{-3}$ *M*.

Solute	Mg^{2+}	Ni^{2+}	Zn^{2+}	Cu^{2+}	k_0'*
Adenine	4.90	4.79	4.91	>30	5.27
cAMP	10.06	8.99	9.46	9.52	9.65
Guanosine	6.21	5.90	6.12	6.38	6.79
Cytosine	0.51	~0.50**	0.53	0.61	0.55
Cytidine	1.18	~1.28**	1.28	1.38	1.38
cCMP	2.50	~2.46**	2.50	–	2.49
Uracil	0.80	~0.75**	0.80	0.80	0.80
Uridine	1.84	1.90	2.01	2.01	2.18

* Capacity ratios with mobile phase not containing metal ions.
** Estimated from values of k' at $1 \cdot 10^{-3}$ and $2.5 \cdot 10^{-3}$ *M* Zn or Ni.

with a mobile phase not containing metal ions was NTP > NDP > NMP. This is the opposite of the order found with phosphate buffers, or in Horváth *et al.*'s work[19] at higher ionic strength (0.1). Addition of Mg(II) ions caused a decrease in k' values and a retention order reversal, as shown in Fig. 1. The same behaviour was found for all the nucleotides studied here. The results of decreasing k' with increasing Mg(II) concentration are similar to those reported previously[20], but are different to those reported by Horváth *et al.*[19]. It was expected that Mg(II) ions would neutralize the phosphate charges and cause an increase in k' values. This was not so, and the reasons for the decrease are not clear to us. The presence of the Mg(II) ions changed the retention of any NTP the most. This is not surprising as the Mg(NTP) complex is more stable than the di- or monophosphate complexes.

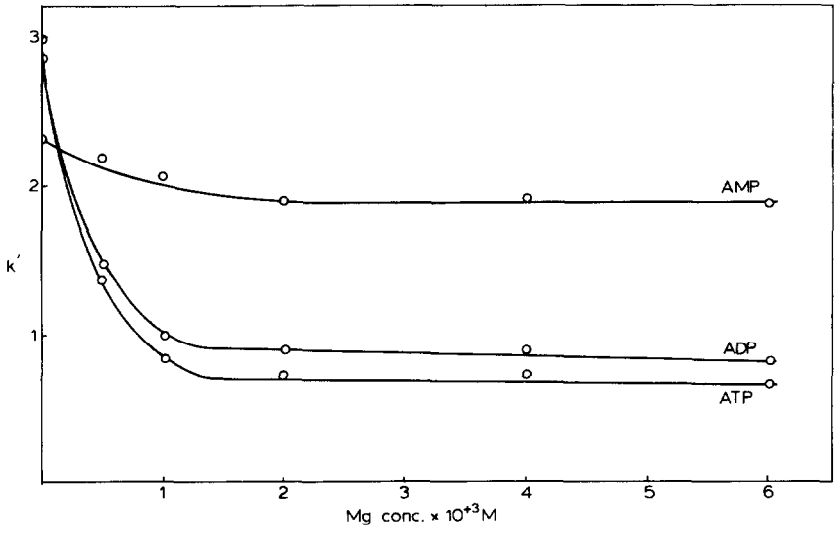

Fig. 1. Dependence of k' on Mg(II) concentration for adenosine nucleotides. Mg(II) in acetate buffer (pH 5.6), temperature = 37°C. Concentrations of nucleotides were $1 \cdot 10^{-3}$ *M*.

Examination of the data in Table I shows that the relative dependence of k' on Mg(II) concentration is roughly the same for all NTPs or NDPs or NMPs. This can be explained by the coordination of Mg(II) ions only to the phosphate group. Hence the nature of the base, and not relative charges, controls the magnitude of k'. The present results substantiate other published works, which show that magnesium ions coordinate to the phosphate groups and not to the base[23,24].

When cations other than Mg(II) are present in the mobile phase the retention behaviour of the nucleotides is different. Figs. 2 and 3 show the dependence of k' for ATP and UTP on the concentration of four different cations. The behaviour shown is typical of all the nucleotides studied here. The opposite effect of Cu(II) and Mg(II) should be noted. With Zn(II) or Ni(II) the behaviour of k' is more complicated. Initial studies were made in the cation concentration range $5 \cdot 10^{-4}$–$5 \cdot 10^{-3}$ M. Figs. 2 and 3 show that in this range the capacity ratios increase with increasing concentration of Zn(II) or Ni(II). However, the k' values are smaller than k'_0 at all concentrations. As extrapolations of such behaviour to zero metal ion content would give capacity ratios smaller than k'_0, an investigation at low concentrations was made. Fig. 2 shows the behaviour of ATP in the presence of $5 \cdot 10^{-5}$, $1 \cdot 10^{-4}$ and $4 \cdot 10^{-4}$ M Ni(II). Table I shows the data for all the adenosine nucleotides and the guanosine nucleotides. It can be seen that at very low concentrations of Ni(II), the capacity ratios decrease. The change in the dependence of k' on the concentration occurs around $4 \cdot 10^{-4}$–$5 \cdot 10^{-4}$ M Ni(II). At $5 \cdot 10^{-5}$ M Ni(II) the retention order is still NMP > NDP > NTP. The change in the retention order occurs between $5 \cdot 10^{-5}$ and $1 \cdot 10^{-4}$ M Ni(II). This behaviour probably holds true with Zn(II) ions also.

At cation concentrations of $2 \cdot 10^{-3}$ M the retention order is Cu–Nu > Zn–Nu > Ni–Nu > Mg–Nu, where Nu represents the nucleotides. The metal–nucleotide complex formation constant (K_f) decreases in the order Cu > Ni > Zn > Mg. Recent K_f compilations[25,26] show that the values for the Zn(II) and Ni(II) complexes

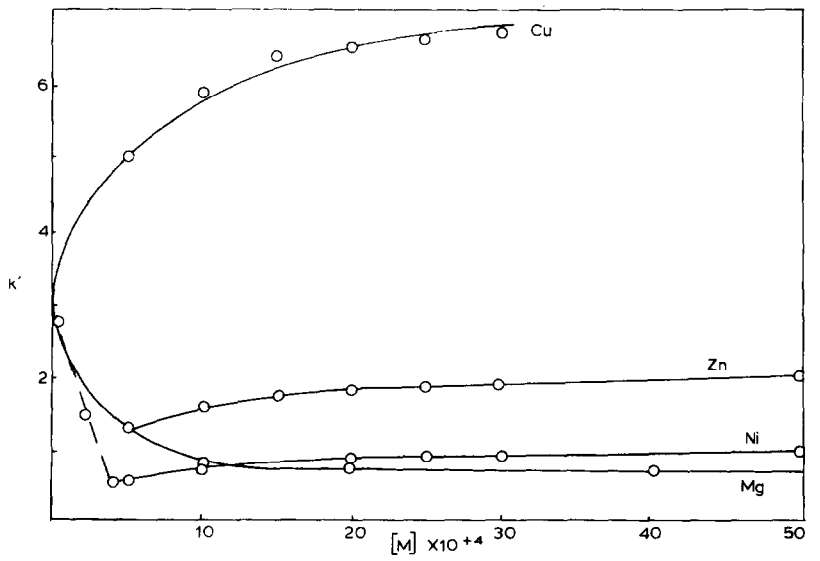

Fig. 2. k' values of ATP as a function of metal ion concentrations. Cations in acetate buffer (pH 5.6).

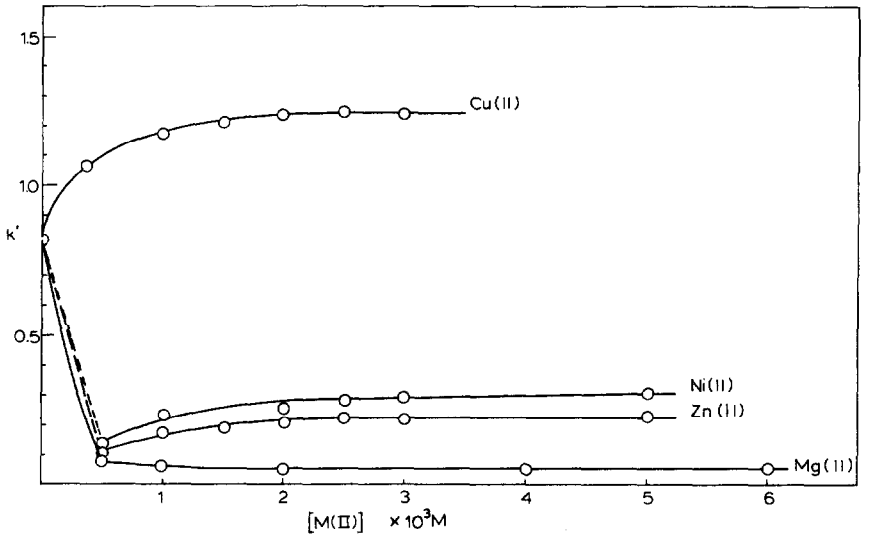

Fig. 3. k' values of UTP as a function of cation concentrations. Conditions as in Fig. 2.

are similar, the former often being slightly larger. This agrees well with the present data, which show that the Ni(II) and Zn(II) complexes behave very similarly, with the Zn(II) complexes having slightly longer analysis times. It should be pointed out that the behaviour depicted here is in agreement with "softness" parameter order when hydration is taken into account[27]. Whether this agreement is fortuitous is not clear to us, especially as in the present instance at least one of the water ligands is replaced with a nucleotide.

Retention and intramolecular associations

As mentioned, metal cations are known to be coordinated by nucleotides. Moreover, different metals are coordinated to different functional groups of the nucleotide[11,15,23]. Thus, Mg(II) ions are thought to be coordinated by the phosphate groups only, whereas Cu(II) can also interact with the base, at least with purines. Zinc and nickel behave in a similar manner to copper, although their interactions with the base are weaker. The interactions with a base can be inter- or intramolecular[23], depending on whether the nucleotide concentration is high or low, and on the type of nucleotide and cation. Magnesium ions promote NTP self-association mainly by charge neutralization. Copper ions, at least with purines, can bridge between two nucleotides, by coordinating to the phosphates of one and N-7 of the other. At very low concentrations of the nucleotides and cations, the M (NTP) complex is in a monomeric form, and Zn, Ni or Cu, but not Mg, can form intramolecular macrochelates, *e.g.*, between phopsphate and the base of the same molecule. Scheller *et al.*[23] found that the NMR chemicasl shifts of Cd(II) and Zn(II) complexes did not behave monotonically as a function of the cation concentration. They attributed this difference to a change from predominantly intermolecular self-association at high concentrations to monomeric intramolecular macrochelates at

low concentrations. In this work the concentration range was such that the monomeric form probably dominated.

As more Cu(II) ions are added, a larger amount of the nucleotide solute is complexed and is in the "closed" form. Mg(II) ions, probably coordinated solely by the phosphate moieties, are not able to promote macrochelation. The Mg(II) is probably hydrated and it appears that the complex is more solvated than the copper complex. The retention behaviours of the Mg(II) and Cu(II) complexes are also entirely different, as shown in Figs. 2 and 3. As discussed in the Introduction, these two complexes have other differing properties and behaviours, *e.g.*, the effect of Mg(II) and Cu(II) on the melting of polynucleotides and DNA[7]. Moreover, the enthalpy of formation of the Mg(II) complex is positive whereas it is negative for the Cu(II) and also the Ni(II) and Zn(II) complexes[28,29]. Therefore, factors that control the retention can be related to factors that affect other physico-chemical properties of the complexes.

At very low concentrations of Zn(II) or Ni(II) the complexes have a similar retention behaviour to the Mg(II) complex. At relatively higher Zn(II) or Ni(II) concentrations (*ca.*. $1 \cdot 10^{-3}$ *M*) the behaviour is similar to that of the Cu(II) complex. The chromatographic data indicate that at low concentrations of zinc and nickel the complex is in the "open" form, whereas at high concentrations the macrochelate form is significant. This point remains to be proved. It should be pointed out that a change in the k' behaviour with concentration of these two cations occurs at an [NTP]:[M] ratio of about 1.

A relationship between the capacity ratio and the extent of macrochelate formation is shown in Table III for ATP. As expected, k' is greater when the concentration of the "closed" form is large. The trends shown in Table III apply not only to ATP but also to all nucleotides for which K_l values could be found.

TABLE III

RELATIONSHIP BETWEEN k' AND RING CLOSURE

[ATP] = $2 \cdot 10^{-3}$ *M*.

Solute	k'*	K_l**	*M(NTP)Cl (%)***	K_f**
Mg(ATP)	0.74	0–0.6	0–38	4.27
Ni(ATP)	0.92	1.8 (2.9)	64 (74)	4.88
Zn(ATP)	1.82	1.6	62	5.19
Cu(ATP)	6.71	3.2	76	6.24

* Extrapolated to limiting value.

** Data from ref. 23 are in parentheses. K_l is the "closed" formation constant. K_f is the M(NTP) formation constant.

For the sake of completion, Table III also gives the complex formation constants, K_f. The relationships between the capacity factors and K_f values are not surprising; in fact they are well known[19,20].

The nature and properties of the metal bond are greatly affected by the nucleotide. One of the prevailing thoughts is that metal–base interactions occur principally in the case of purine nucleotides. Using current values[23] for intramolecular

chelate formation, Fig. 4 shows normalized k' values *versus* the fraction of complex in the "closed"' form, for the four Cu(NTP) complexes studied here. The CTP complex seems to be out of place. This is probably due to a small error in determining the k'_0 value.

In Fig. 4 it was assumed that all the cations interact only with the phosphates in the case of CTP and UTP. However, Fig. 3 and the complete set of data indicate that some intramolecular macrochelations do perhaps take place, albeit to a small extent, even with these two nucleotides. This point must be further investigated.

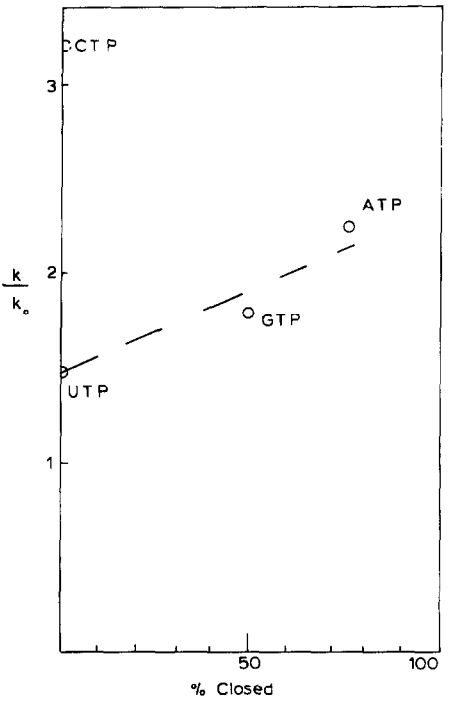

Fig. 4. Relationsship between the retention and the extent of intramolecular macrochelation, *i.e.*, the closed form. k'_0 is the capacity factor in the absence of metal cations in the mobile phase.

The above discussion is self-consistent with the observations of Brown and Grushka[21]. Stacking depends, in the case of metal–nucleotide complexes, on the interaction induced by the cations. Efficient self-association requires strong interactions between one monomer with its neighbours. Metal ions that promote intramolecular interaction can, under the right conditions, mediate in stacking. In other words, factors affecting stacking can affect the retention.

The adsorption of the metal cations by residual silanol groups is a distinct possibility[19]. Such adsorption will, of course, be manifested in the retention behaviour of the nucleotides. It is felt, however, that the presence of free silanols and the possible adsorption of the cations does not explain the decrease in retention when Mg(II), Ni(II) or Zn(II) cations are added to the mobile phase. The nucleotide–cation complex is fairly stable and it could also be formed on the stationary phase. Thus

adsorbed cations will increase the retention of the nucleotide, in a similar manner to free cations in the mobile phase. The determination of K_f would require an exact knowledge of the possible adsorption of the metal cations. This, however, was not the purpose of this work. The trend of the data as discussed above and in the following sections, of course, is independent of the presence of silanols.

Correlations between retention and physico-chemical properties

Chromatographic data can be used to study some physico-chemical properties of the complexes. For example, Table IV shows the relative changes in the capacity ratios and in base acidities for metal complexes of GTP and of UTP (the order clearly follows the order of formation constants). One can use retention data to predict, if only in a qualitative manner, the change in proton acidity of N-1 in purines or N-3 in pyrimidines, and *vice versa*. It is not surprising that the two parameters are interrelated. The retention time is a function of, among other factors, the complex formation. Changes in base acidity indicate electronic changes that are also due to the formation of the complex.

TABLE IV

COMPARISON BETWEEN $\%\Delta k'$ OF GTP AND UTP AND CHANGES IN BASE ACIDITY CONSTANTS

Solute	ΔpK_a*	$\%\Delta k'$**
Mg(GTP)	0.2	−89
Ni(GTP)	1.15	−76
Zn(GTP)	1.40	−62
Cu(GTP)	1.9	+74
Mg(UTP)	0.2	−94
Ni(UTP)	0.60	−69
Zn(UTP)	0.99	−74
Cu(UTP)	1.7	+53

* Data from ref. 24.

** $\Delta k' = (k' - k'_0)/k_0$. k' measured at $2 \cdot 10^{-3}$ M cation concentration.

Sigel[24] briefly discussed the formation of the hydroxo complex $M(NTP)(OH)^{3-}$. Table V shows the pK^H values of the dissociation

$$M(NTP)(H_2O)^{2-} \rightleftharpoons M(NTP)(OH)^{3-} + H^+$$

for ATP and CTP in the presence of several metal cations, and also relative changes in k'. Again, there is a trend: as the formation of the hydroxo complex increases, the change in k' becomes more positive.

Metal ions are known to accelerate nucleotide dephosphorylation[8,30]. Sigel and Amsler[8] showed that the dephosphorylation rates decrease in the order Cu(NTP) > Zn(NTP) > Ni(NTP), which is similar to the retention order. On the whole, it was found[8] that the effect of metal ions on dephosphorylation was smaller for pyrimidine-based nucleotides than with the purines. The present data show the same trend: in general, the (relative) effect of a metal cation on the k' values was greater

TABLE V

FORMATION OF HYDROXO COMPLEXES AND $\%\Delta k'$

$M(NTP)(H_2O)^{2-} \rightleftharpoons M(NTP)(OH)^{3-} + H^+$.

M	*ATP*		*CPT*	
	pK^{H*}	$\%\Delta k'$**	pK^{H*}	$\%\Delta k'$**
Ni	9.41	− 69	9.58	− 64
Zn	8.89	− 39	8.79	− 58
Cu	7.9	+117	7.6	+221

* Data from ref. 24.

** $\Delta k' = (k' - k'_0)/k'_0$. k' measured at $2 \cdot 10^{-3}$ *M* cation concentration.

with adenosine and guanosine nucleotides than with the cytidine and uridine nucleotides. It is interesting to note in the context of this work that Sigel and Amsler[8] used the concept of metal-bridged stacking to explain the enhancement in dephosphorylation. Once again it is seen that the chromatographic technique can yield information, even if only qualitative, about the nature of metal–nucleotide complexes.

CONCLUSIONS

The importance of metal–base interactions should not be overlooked. Properties such as stacking, dephosphorylation, acidities and chromatographic retentions are affected by such interactions. It is tempting to speculate further about the role of the base and relate it to the functioning of the nucleotide. For example, the availability of N-7 in purines to other types of chemical species is essential for the proper functioning of nucleotides. Therefore, Mg(II) is a viable co-factor and Cu(II) is not, as the former coordinates only with the phosphates leaving N-7 free for other interactions, while Cu(II) can interact strongly with the base, thus "poisoning" the nucleotides. Clearly, such a speculation has to be further investigated. As chromatographic retention seems to be a strong function of metal ion–base interactions, it can serve as a probe in such an investigation.

Chromatography is used, of course, for the separation and purification of nucleotides. However, it can also be employed to ascertain the properties of such solutes and the properties of their metal complexes. This is due to the fact that chromatography is sensitive to charge and structural changes. The method described here can be used to investigate factors such as hydrophobicity and the tendency to form all types of metal complexes such as the ternary amino acid–cation–nucleotide complex. The simplicity by which this information can be attained is very attractive. The disadvantage of the method is that only the overall behaviour of a solute is obtained. This is in contrast to techniques such as NMR, which can probe specific regions in the molecule. However, chromatography allows properties to be studied at very low concentrations, which are unattainable by other techniques. Current work in our laboratory concerning the retention and properties of ternary amino acid–cation–nucleotide complexes, to be reported separately, takes advantage of the feasibility of such low concentrations for characterization purposes.

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