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Metal Ion/Buffer Interactions*. Stability of Alkali and Alkaline Earth Ion Complexes with Triethanolamine (Tea), 2-Amino-2(hydroxymethyl)-1,3-propanediol (Tris) and 2-[Bis(2-hydroxyethyl)-amino] 2(hydroxymethyl)-1,3-propanediol (Bistris) in Aqueous and Mixed Solvents

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The acidity constants of the protonated buffers given in the title, i.e. *of $H(Tea)^+$, $H(tris)^+$ and $H(Bistris)^+$, have been measured at 25 °C in water, 50% aqueous dioxane or methanol, and in 75 or 90% dimethylsulfoxide (Dmso) with tetramethylammonium nitrate as background electrolyte. The interaction of Tea, Tris, or Bistris (L) with the alkali or alkaline earth ions (M^{n+}) was studied by potentiometric pH titrations in the same solvents and the stability constants of the ML^{n+} complexes were determined. The stability constants, log K^{M}_{ML}, of the Na^+ complexes with the several buffer-ligands in the given solvents vary from −1.05 [$Na(Tea)^+$ in H_2O; $I = 1.0$] to 0.54 log units [$Na(Bistris)^+$ in 90% Dmso; $I = 0.25$]; the corresponding values for the Mg^{2+} complexes range from 0.24 [$Mg(Tea)^{2+}$ in H_2O; $I = 1.0$] to 0.91 log units [$Mg(Bistris)^{2+}$ in 90% Dmso; $I = 0.25$]. Unexpectedly, $Ca(Bistris)^{2+}$ is the most stable among the alkaline earth ion complexes in aqueous solution (log $K^{Ca}_{Ca(Bistris)} = 2.25$; the corresponding values for the Mg^{2+}, Sr^{2+} and Ba^{2+} complexes are 0.34, 1.44 and 0.85, respectively; $I = 1.0$), while in 90% Dmso $Sr(Bistris)^{2+}$ is most stable (log $K^{Sr}_{Sr(Bistris)} = 1.87$; the corresponding values for the Mg^{2+}, Ca^{2+} and Ba^{2+} complexes are 0.91, 1.64 and 1.14, respectively; $I = 0.25$). A similar, but less pronounced pattern is observed for the $M(Tea)^{n+}$ complexes. Obviously, the stabilities of the alkaline earth ion complexes with Bistris and Tea follow neither the order of the ionic radii nor that of the hydrated radii of the cations. In contrast, in all solvents the stability of the alkali ion complexes increases with decreasing ionic radii; this being also true for the alkaline earth ion complexes of Tris in aqueous solution. The possible reasons for these observations, the structures of the complexes in solution, and some biological implications are discussed. Calculations of the extent of complex formation show that in the physiological pH range the concentration of certain complexes may be quite pronounced; hence reservations should be exercised in employing these buffers in systems which also contain metal ions.*

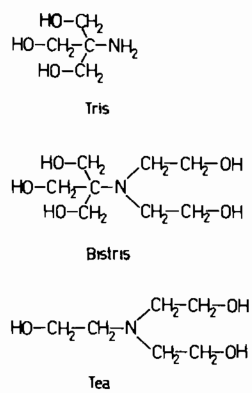

Fig. 1. Chemical formula of 2-amino-2(hydroxymethyl)-1,3-propanediol (Tris), 2-[bis(2-hydroxyethyl)amino]-2(hydroxymethyl)-1,3-propanediol (Bistris) and triethanolamine (Tea).

Introduction

The acidity constants, pK_A, of the monoprotonated species of triethanolamine (Tea), 2-amino-2(hydroxymethyl)-1,3-propanediol (Tris) and 2-[bis-(2-hydroxyethyl)amino]-2(hydroxymethyl)-1,3-propanediol (Bistris) in aqueous solution at room temperature are between 6.5 and 8.3 [1, 2]. Because the buffer region of these compounds, whose structures are shown in Fig. 1, cover the physiological pH range, they are often used in biochemical studies. Tris is an especially favored buffer [3, 4], and Bistris [5] is also increasingly used [6].

All pH buffers contain a basic site, in the present cases an amino nitrogen, and therefore they are

*This is part 3 of the series; for parts 1 and 2 see [1] and [2], respectively.

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potential ligands for metal ions. Indeed, the interference of buffers in reactions where metal ions participate are well known. For example the activity of pyruvate kinase, a Mg^{2+}-containing enzyme, is inhibited by Tris [7] as is the metal ion promoted dephosphorylation of ATP [8]*, while alkaline phosphatase, a Zn^{2+}-containing enzyme, was found to be activated by Tris buffer [9]. Aside from the mild reducing nature [10, 11] and the cationic effect of HL^+ [12, 13], the most likely interference in these systems is that the buffer in its neutral form undergoes complex formation with the metal ions present.

Complexes between transition metal ions and Tris [1, 14–16], Bistris [2] or Tea [14–16] are well known and their stability in aqueous solution has been determined. The corresponding complexes with the alkali cations have not been studied, and for those of the alkaline earth ions only limited stability data are available for Bistris [2], together with the upper limits of the stability of some Tris complexes [1]. As alkali and alkaline earth ion complexes play an important role in nature [17–20], and as *e.g.*, Bistris has been recommended as part of buffer combinations for mammalian cell cultures [21], which also contain Na^+, K^+, Mg^{2+} and Ca^{2+} [22], it seemed appropriate to study the metal ion coordinating properties of the structurally related buffer-ligands of Fig. 1 towards alkali and alkaline earth ions in some detail. This was also appealing in a general sense, because the thermodynamic properties of alkali complexes in aqueous solution have been studied relatively little [14–16, 23, 24], although such complexes have been known for a long time [25] and macrocycle-alkali metal cation interactions receive much interest at present [26–29]. To learn how the polarity of the solvent or a reduced water activity influences complex stability, the complexes have also been studied in mixed aqueous solvents containing methanol, dioxane, dimethylsulfoxide (Dmso) or dimethylformamide (Dmf).

Experimental

Materials

The nitrate salts of Li^+ (suprapur), Na^+, K^+, Mg^{2+}, Ca^{2+}, Sr^{2+} and Ba^{2+}, a 10% tetramethylammonium hydroxide solution (which was converted into the nitrate), HNO_3 (p.a.), triethanolamine hydrochloride (p.a.) and the solvents, dioxane, methanol, Dmso and Dmf were from Merck AG, Darmstadt, Germany. $RbNO_3$ was obtained from Alfa Division, Ventron Corporation, Danvers, Mass., U.S.A. The free base of Bistris (p.a.) was from Serva Feinbiochemica GmbH, Heidelberg, Germany, and the nitrate salt of Tris (reagent grade) from Sigma Chemical Co., St. Louis, Missouri, U.S.A.

The exact titer of the hydroxide solutions used for the titrations was determined with potassium hydrogen phthalate (Merck AG). The stock solutions of the alkali salts were prepared by weighing the calculated amount of salt, while the exact concentrations of the alkaline earth ion stock solutions were determined with Edta (Merck AG). The exact concentration of the stock solutions of the ligands (buffers) was also determined by titration.

Determination of Equilibrium Constants by Potentiometric Titrations

The titrations were carried out with a Metrohm potentiograph E536 and a Metrohm macro EA121 glass electrode (25 °C). The buffers (pH 4.64, 7.00 and 9.00) used for calibration were also from Metrohm AG, Herisau, Switzerland. The direct readings for pH were used in the calculations; no 'corrections' were applied for the change in solvent, *e.g.*, to 50% aqueous dioxane (*cf.* [30]).

The acidity constant K^H_{HL} of $H(Bistris)^+$ was determined by titrating 25 ml 0.0017 *M* HNO_3 and $Tma^+NO_3^-$ (I = 0.25, 0.5 or 1.0 depending on the solvent; 25 °C) in the presence and absence of 0.0015 *M* Bistris under N_2 with 1 ml 0.05 *M* tetramethylammonium hydroxide solution (Tma^+OH^-). The acidity constants of $H(Tris)^+$ and $H(Tea)^+$ (0.0015 *M*) were determined in exactly the same way, but HNO_3 was only 0.0003 *M*. K^H_{HL} was calculated within the range between 3% and 97% neutralization, where possible.

The conditions for the determination of the stability constants K^M_{ML} (I = 0.25, 0.5 or 1.0 depending on the solvent; 25 °C) were the same as for the acidity constants, but $Tma^+NO_3^-$ was (usually completely) replaced by M^INO_3 (0.25, 0.5 or 1.0 *M* depending on the desired ionic strength) or $M^{II}(NO_3)_2$ (0.083, 0.167 or 0.333 *M*). Hence, the ratios of M^{n+}:L were between about 55:1 and 667:1, *i.e.* under these conditions only 1:1 complexes form and species $M(L)_m$ with m $\geqslant$ 2 may be neglected. The stability constants K^M_{ML} were computed by taking into account the species H^+, HL^+, L, M^{n+} and ML^{n+} [31], and the data were usually collected from 10% complex formation on. Hydrolysis of M^+_{aq} or M^{2+}_{aq} did not interfere, as was evident from the titrations without L.

The equilibrium constants listed in Table I were usually calculated from three independent titration

*Abbreviations aside from those given in Fig. 1: ADP, adenosine 5′-diphosphate, ATP, adenosine 5′-triphosphate; Dmf, dimethylformamide; Dmso, dimethylsulfoxide; L, general (buffer-) ligand; M^{n+}, general metal ion, Tma^+, tetramethylammonium ion.

curves. The errors given are three times the standard error of the mean value or the sum of the probable systematic errors, whichever is larger.

Results and Discussion

1. Basicity of the Buffer-Ligands

Tris, Bistris and Tea are buffers with an amino nitrogen as their basic site. To reduce any undesired interaction between these potential ligands and other species present in solution the ionic strength was kept constant by using tetramethylammonium nitrate ($Tma^+NO_3^-$) as background electrolyte. For Tris [32, 33] and Bistris [34] it is known that their acidity constant shows a relatively large temperature dependence. The present potentiometric pH titrations were carried out at 25 °C and the acidity constants were calculated according to equilibrium 1

$$HL^+ \rightleftharpoons H^+ + L \qquad K^H_{HL} = [H][L]/[HL] \qquad (1)$$

It has been observed [2] that the presence of hydroxy groups two C atoms distant from the basic N leads to a systematic decrease of the basicity which is fairly independent of the type of amine (*i.e.* primary, secondary, or tertiary). Each hydroxy group lowers pK^H_{HL} by about 0.8 log units. In agreement herewith the acidity constants in aqueous solution (I = 1.0; 25 °C) of $H(Tris)^+$ (pK^H_{HL} = 8.31) and $H(Tea)^+$ (8.05) with their three hydroxy groups are rather similar, while $H(Bistris)^+$ with its five hydroxy groups is considerably more acidic ($pK^H_{H(Bistris)}$ = 6.74). This implies that for all these hydroxy groups it is sterically possible to reach the vicinity of the basic nitrogen; with regard to metal ion complexes this is a hint that these hydroxy groups might participate in complex formation.

Increasing ionic strength increases the basicity of Bistris slightly as is evident from the results obtained in 50% aqueous dioxane: $pK^H_{H(Bistris)}$ = 6.72 (at I = 0.5) < 6.80 (at I = 1.0). This agrees with earlier observations made in aqueous solution for Bistris [2], and Tris [1] and NH_3 as well [35]. Hence, with decreasing water activity the release of the proton from the amino nitrogen is rendered more difficult.

In this connection a more general consideration [36] seems appropriate: The dissociation of a *neutral* species into two charged species is expected to be inhibited if less water for solvation of the charged species becomes available. Indeed, *e.g.*, for CH_3COOH holds $pK^H_{H(Ac)}$ = 4.54 in water (I = 0.1; 25 °C) [37] and 6.01 in 50% aqueous (v/v) dioxane (I = 0.1; 25 °C) [38, 39]. In contrast to this, one would expect that the dissociation of a *protonated* species as in equilibrium 1 is less influenced by a change in the solvent (because on both sides of the equilibrium charged species occur), or that deprotonation is even facilitated because the organic part of the solvent mixture may facilitate solvation of the uncharged species L. Indeed, *e.g.*, for ammonia holds $pK^H_{NH_4}$ = 9.36 in water [37] and 8.91 in 50% aqueous (wt) methanol [40] (I = 0.1; 25 °C), and for γ-picoline holds $pK^H_{H(Pc)}$ = 6.18 in water and 5.11 in 50% aqueous (v/v) dioxane (I = 0.1; 25 °C) [41].

The properties of $H(Bistris)^+$ at I = 0.5 correspond approximately to this expectation [$pK^H_{H(Bistris)}$ = 6.65 in water [2], 6.72 in 50% aqueous dioxane, and 6.61 in 50% aqueous methanol], *i.e.* the influence of these mixed solvents on the acidity constants is small. However, the solvent-change to 75 or 90% Dmso (I = 0.25) *increases* the basicity of Bistris considerably, $pK^H_{H(Bistris)}$ = 7.26 or 7.47, respectively; this may indicate that under these conditions in $H(Bistris)^+$ the proton is *intra*-molecularly 'solvated' by the hydroxy groups. In other words, it appears that hydrogen bonds are formed. The same effect is also observed with $H(Tea)^+$, but much less pronounced [$pK^H_{H(Tea)}$ = 8.05 in water (I = 1.0) and 8.24 in 90% Dmso (I = 0.25)], while with $H(Tris)^+$ it is rather dramatic: the basicity increases by about 1.8 log units [$pK^H_{H(Tris)}$ = 8.31 in water (I = 1.0) and 10.11 in 90% Dmso (I = 0.25)]. This may indicate that the 2(hydroxymethyl)-1,3-propanediol moiety is especially effective in such an *intra*molecular 'solvation', although the formation of the rather symmetrical $-NH_3^+$ group, which is ideally suited for hydrogen bonding may play a role as well.

2. Stability of the $M(Buffer)^{n+}$ Complexes

From potentiometric pH titrations it is immediately obvious that Tris, Bistris and Tea form rather stable complexes with several alkali or alkaline earth cations, because in their presence the buffer regions are significantly shifted towards lower pH. All observations could be fully characterized by the following equilibrium:

$$M^{n+} + L \rightleftharpoons ML^{n+} \qquad K^M_{ML} = [ML]/([M][L]) \qquad (2)$$

The stability constants determined for the $M(buffer)^{n+}$ complexes in aqueous solution and in several mixed solvents* are summarized in Table I.

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*The following results in 65% (v/v) aqueous dimethylformamide (this corresponds to 0.30 mole fraction of Dmf) indicate that in this solvent (I = 0.5, $Tma^+NO_3^-$; 25 °C) complex formation with Bistris is promoted, *i.e.* even somewhat more than in 90% Dmso. However, the titration curves showed a considerable drift in dependence on pH; therefore the following values can only be considered as rough estimations: $pK^H_{H(Bistris)} \cong 7.4$, $\log K^M_{M(Bistris)}$ for $Li^+ \cong 0.9$, for $Na^+ \cong 0.7$, for $K^+ \cong 0.5$, and for $Rb^+ \cong 0.5$.

TABLE I Negative Logarithms of the Acidity Constants of Protonated Triethanolamine (Tea), 2-Amino-2(hydroxymethyl)-1,3-propanediol (Tris) or 2-[Bis(2-hydroxyethyl)-amino]-2(hydroxymethyl)-1,3-propanediol (Bistris) and Logarithms of the Stability Constants of the Alkali and Alkaline Earth Ion Complexes with the Mentioned Buffers in Several Solvents at 25 °C.* The Values given in *Italics* are normalized for the Stability of Li(Bistris)$^+$ in Water (*cf*)[a].

Solvent	I	Inert NO_3^- Salt[b]	Ligand(L)	pK^H_{HL}	Log K^M_{ML}							
					Li^+	Na^+	K^+	Rb^+	Mg^{2+}	Ca^{2+}	Sr^{2+}	Ba^{2+}
H_2O	1.0	Tma^+/K^+	Tris	8.31 ± 0 01[c]	−0.23 ± 0.03	−0 72 ± 0.01	d,e	d,e	0 30 ± 0.01	0 25 ± 0.02	0.11 ± 0.02	0 02 ± 0.02
					1.1	*0 36*			*3.8*	*3 4*	*2.5*	*2 0*
H_2O	1.0	Tma^+/K^+	Tea	8.05 ± 0.01	−0.48 ± 0.05	−1.05 ± 0 12	d,f	d,f	0.24 ± 0.02	0.78 ± 0.01	0.38 ± 0 01	0.36 ± 0.01
					0.63	*0 17*			*3.3*	*11*	*4.6*	*4.4*
H_2O	1.0	Tma^+/K^+	Bistris	6.74 ± 0.01[g]	−0.28 ± 0.01	−0.82 ± 0.03	d,e	d,e	0.34 ± 0.05[h]	2.25 ± 0 02[h]	1 44 ± 0.02[h]	0.85 ± 0.03[h]
					1	*0 29*			*4 2*	*340*	*52*	*13*
50% Dioxane	1.0	Tma^+	Bistris	6.80 ± 0 01	0.12 ± 0.01	−0.57 ± 0.04	i	i				
(0.17)[j]					*2.5*	*0.51*						
50% Dioxane	0.5	Tma^+/K^+	Bistris	6.72 ± 0.01	0.15 ± 0.01	−0.34 ± 0.02	d,k	d,k	0.51 ± 0.02	2.91 ± 0.01	2.24 ± 0 01	1.33 ± 0 01
(0.17)[j]					*2.7*	*0.87*			*6.2*	*1500*	*330*	*41*
50% CH_3OH	0 5	Tma^+/K^+	Bistris	6 61 ± 0.01	0.08 ± 0 09	−0.39 ± 0.03	d,k	d,k	0 43 ± 0.01	2.94 ± 0.01	2 22 ± 0 01	i
(0.31)[j]					*2.3*	*0 78*			*5 1*	*1700*	*320*	
75% Dmso	0.5	Tma^+	Bistris	7.26 ± 0 01	0.34 ± 0.02	0.06 ± 0 03	−0.47 ± 0.14	d,k	0 44 ± 0.01	2.16 ± 0 02	2.19 ± 0.01	1 25 ± 0.01
(0.43)[j]					*4 2*	*2.2*	*0 65*		*5.2*	*280*	*300*	*34*
90% Dmso	0.25	Tma^+	Bistris	7.47 ± 0.01	0.61 ± 0.01	0.54 ± 0.01	0.36 ± 0.01	0.26 ± 0.01	0.91 ± 0.04	1.64 ± 0.01	1.87 ± 0 01	1.14 ± 0.03
(0.70)[j]					*7.8*	*6.6*	*4.4*	*3.5*	*15*	*83*	*140*	*26*
90% Dmso	0.25	Tma^+/Rb^+	Tris	10.11 ± 0.01	0.37 ± 0.03	0.29 ± 0.05	−0.24 ± 0.10	d,l	0.50 ± 0.22	0.25 ± 0.10	0 76 ± 0.06	0.41 ± 0.02
(0.70)[j]					*4.5*	*3 7*	*1 1*		*6 0*	*3.4*	*11*	*4 9*
90% Dmso	0.25	Tma^+	Tea	8.24 ± 0.01	0.49 ± 0.03	0.47 ± 0.03	0 07 ± 0.07	−0.07 ± 0.13	0.51 ± 0.03	0.82 ± 0.02	0.80 ± 0.02	0.58 ± 0.04
(0.70)[j]					*5.9*	*5.6*	*2.2*	*1.6*	*6 2*	*13*	*12*	*7.2*

*See footnote p. 149. [a]This means the ratio $K^M_{ML}/K^{Li}_{Li(Bistris)}$ is given, where $K^{Li}_{Li(Bistris)} = 10^{-0.28} = 0.525$. [b]If in comparison with Tma^+ no depression of the buffer region was observed with K^+ or Rb^+, *i.e.* no complex formation with K^+ or Rb^+ was detected, then in the titrations of the alkaline earth ions KNO_3 or $RbNO_3$ was used for adjusting the ionic strength. [c]This value agrees well with $pK^H_{H(Tris)}$ = 8 33 ± 0.01 (25 °C; I = 1 0, KNO_3) of [1]. [d]No depression of the buffer region, relative to the titrations in the presence of Tma^+, was observed with these ions, but the upper limits of log K^M_{ML} may be estimated (*cf* [e,f,k,l]). [e]Assuming a depression of the buffer region of 0 05 log units (Δ pH) would have been recognized one obtains log $K^M_{ML} \leq -0.9$. [f]For Δ pH = 0 04 (see[e]) one obtains log $K^M_{M(Tea)} \leq -1.0$. [g]This result agrees well with $pK^H_{H(Bistris)}$ = 6.72 ± 0.01 (25 °C, I = 0.1, KNO_3) of [2]. [h]From [2]. [i]The corresponding nitrate salt was not enough soluble under these conditions. [j]The percentages are given as v/v. The number in parenthesis is the mole fraction of the organic part of the aqueous solvent mixture. [k]For Δ pH = 0 05 (see[e]) one obtains log $K^M_{ML} \leq -0$ 6. [l]For Δ pH = 0.05 (see[e]) one obtains log $K^M_{ML} \leq -0.3$.

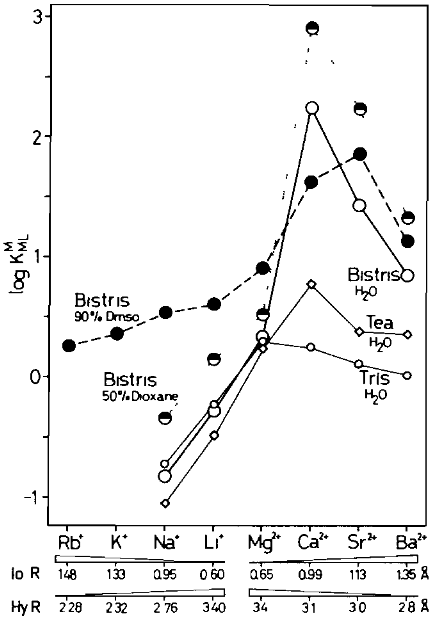

Fig. 2. Plots of the stability constants log K^M_{ML} for the alkali and alkaline earth ion complexes with Tea (◇), Tris (○) and Bistris (○,◓,●) in aqueous solution (◇, ○, ○), in 50% aqueous dioxane (◓), and in 90% aqueous Dmso (●); the plotted data are taken from Table I The ionic radii (= Io.R.) (according to Pauling) are from [44], the approximate hydrated radii (= Hy.R.) of the alkali cations are also from [44] while those of the alkaline earth ions are from [45]. The wedge-shaped notations indicate the trends of the radii.

2.1. Complexes with Alkali Ions

The stability of the alkali cation complexes with all three buffers is usually weak and the stability order is $Rb^+ < K^+ < Na^+ < Li^+$, in accordance with conductance studies of M^+/Tea systems in tetrahydrofuran [42], and the stability of alkali complexes of carboxylates and hydroxycarboxylates in aqueous solution [43]. This means that complex stability increases with decreasing ionic radii of the alkali ions. The stability constants (eqn. 2) of the Na^+, Li^+ or Mg^{2+} complexes of Tris, Bistris and Tea in aqueous solution are for each metal ion remarkably similar, as is evident from Fig. 2 and from the 'normalized' stability values ($K^M_{ML}/K^{Li}_{Li(Bistris)}$) given in *italics* in Table I. This is the reason why the complexes of Li^+ and Mg^{2+} have been plotted next to each other in Fig. 2; indeed, the well-known diagonal relationship within the periodic table predicts similarities for Li^+ and Mg^{2+}.

It should also be added that for $Li(NH_3)^+$ log $K^{Li}_{Li(NH_3)} = -0.3$ [46]; this value is nearly the same as the corresponding constants of the three buffer complexes (Table I). However, as soon as the different basicity of the ligands is also taken into account [2] by considering equilibrium 3

$$M^{n+} + HL^+ \rightleftharpoons ML^{n+} + H^+$$

$$K_{M/HL} = [ML][H]/([M][HL]) \quad (3)$$

and the corresponding constant $pK_{M/HL}$, calculated according to eqn. 4,

$$pK_{M/HL} = pK^H_{HL} - \log K^M_{ML} \quad (4)$$

it becomes evident that the coordinating properties of these ligands towards Na^+, Li^+ and Mg^{2+} decrease in aqueous solution in the order Bistris > Tris ~ Tea > NH_3. This means that complex stability depends on the number of available hydroxy groups, indicating that these groups participate in complex formation.

A change in the solvent from water to 90% aqueous Dmso favors the stability of the alkaline and Mg^{2+} complexes with all three buffers, but it especially favors the stability of the Bistris complexes (Table I). This becomes even more evident if the different ligand basicities are considered (eqns. 3 and 4): complex stability increases within the series $Rb^+ < K^+ < Na^+ < Li^+ < Mg^{2+}$ as expected, and decreases for the series Bistris > Tea > Tris. Due to the solvent change there is a discrimination in complex stability between Tea and Tris indicating that the structure of Tea is more suitable for a coordination of all donor atoms.

From the additional results with Bistris it follows that the change in solvent from water to 50% aqueous dioxane or 50% aqueous methanol alters the basicity of the ligand only a little, while the stability of the alkali cation complexes clearly increases (Fig. 2).

2.2. Alkaline Earth Ion Complexes

From Table I and Fig. 2 several points about complex stability in aqueous solution become obvious: (i) the stability constants of the complexes with Na^+, Li^+ and Mg^{2+} are quite similar for all three buffers, while they differ for the complexes with Ca^{2+}, Sr^{2+} and Ba^{2+}. (ii) Complex stability with Bistris is much higher for these latter mentioned alkaline earth ions than for the corresponding complexes with Tea and Tris, this holds also if eqn. 4 is considered. (iii) For Tea and Bistris the stability of the Ca^{2+} complexes is higher than that of the neighboring ions.

The observed order in stability for the Tris complexes, $Mg^{2+} > Ca^{2+} > Sr^{2+} > Ba^{2+}$ corresponds to the observations made with the alkali complexes, *i.e.* this is the order expected from the ionic radii of the cations (*cf.* Fig. 2). The same order in stability [47] has been observed *e.g.* for the corresponding complexes with oxalate and glycinate [36], and it seems also to hold for the complexes with ammonia: log $K^{Mg}_{Mg(NH_3)} = 0.23 >$ log $K^{Ca}_{Ca(NH_3)} = -0.2$ [46]. The reverse order in complex stability, *i.e.* $Mg^{2+} < Ca^{2+} < Sr^{2+} < Ba^{2+}$, which follows the hydrated radii of the cations, has been observed [48, 49] *e.g.* for inorganic ligands of large dimensions, such as iodate or sulfate [35].

The order in stability $Mg^{2+} < Ca^{2+} > Sr^{2+} > Ba^{2+}$ as determined in aqueous solution for the complexes with Tea and Bistris (Fig. 2) appears as a combination of the two preceding series, and has also been found *e.g.* for the complexes with malate, $^{-}OOCCH_2$-$CH(OH)COO^{-}$ [48]. This result has earlier been explained by us with a 'cage-like' orientation of the hydroxy groups and the nitrogen of Bistris [2], *i.e.* by a structural arrangement into which Ca^{2+} fits well, while the ionic radius of Mg^{2+} is too small and the radii of Sr^{2+} and Ba^{2+} are too large to allow an optimal interaction with the hydroxy groups. This selectivity (especially of Bistris) to complex preferably with Ca^{2+} within the alkaline earth ion series (*cf.* the 'normalized' stability values in Table I) corresponds to observations made with 'crown' ethers [28, 50] and other macrocyclic ligands [26, 27, 29, 51] for which the size of the ligand-cavity is the crucial property.

In the light of the present results, it appears that the given explanation still holds for Mg^{2+} and Ba^{2+}, while the explanation for the observed differences between Ca^{2+} and Sr^{2+} seems more complicated. There are indications that the formation if *intra*-molecular hydrogen bonds between the hydroxy groups and metal ion-coordinated water molecules are also important. This is supported by the following points. (i) Subtle changes in water activity by changing the solvent from water to 50% aqueous dioxane increases complex stability, but does not change the stability order. (ii) A more significant change, *i.e.* to 75% Dmso leaves $Ca(Bistris)^{2+}$ practically unaffected but favors the stability of $Sr(Bistris)^{2+}$ considerably, so that both complexes are of about the same stability in this solvent. Moreover, a change to 90% Dmso changes the order in stability completely, $Mg^{2+} < Ca^{2+} < Sr^{2+} > Ba^{2+}$, *i.e.* the most stable complex is now $Sr(Bistris)^{2+}$ (Fig. 2). (iii) In 90% Dmso the order in stability for the Tris complexes becomes quite 'irregular': $Sr^{2+} > Mg^{2+} \sim Ba^{2+} > Ca^{2+}$. It seems that these observations can be rationalized most easily by *intra*-molecular hydrogen bond formation, because hydrogen bond formation may be favored or disturbed depending on the conditions and the properties of the metal ion. That these ligands have a large tendency to form hydrogen bonds is known, *e.g.*, Tea dimerizes in the crystalline state via a 6-membered ring which results from H-bond bridges [52].

3. On the Structures of the Complexes

Before discussing further the structure of the complexes in solution it seems appropriate to consider first the results of some crystal structural analysis. Solid (Tea)NaI contains seven-coordinated Na^+, *i.e.* each Na^+ is coordinated to the four donor atoms of one Tea, to two oxygens of a neighboring Tea, and to the iodide anion [53]. The distances are Na–I 3.286 Å, Na–N 2.610 Å, and Na–O 2.446 to 2.621 Å. How adaptable the coordination sphere of Na^+ is becomes evident from the X-ray analysis of the complex with O-methylated Tea: $N(CH_2CH_2OCH_3)_3$-NaI [54]. Here Na^+ is only five-coordinated, namely to the four donor atoms of the ligand and to I^-; the distances are Na–I 2.972 Å, Na–N 2.466 Å, and Na–O between 2.339 and 2.373 Å. Hence, the whole coordination sphere with this ligand became smaller.

The molecular structure of $(Tea)_2Sr(NO_3)_2$ contains an approximately cubic eight-coordinated Sr^{2+}, which is surrounded by the eight donor atoms of the two Tea ligands [55]. The two NO_3^- are not linked to the cation, but interact in strong hydrogen bonds with the OH groups of the Tea molecules; the distances are for Sr–N 2.830 Å and for Sr–O 2.534 to 2.594 Å. In a related Ba^{2+} complex, $(Tea)_2Ba(CH_3$-$COO)_2$, the cation has coordination number nine and it is bound to the eight donor atoms of two Tea ligands and to an oxygen of one acetate [56]. The Ba–N distances are 3.025 and 3.108 Å, while the Ba–O distances of Tea are between 2.743 and 2.805 Å, the distance to the O of coordinated acetate is 2.726 Å, while the second acetate is more than 5 Å away. Again hydrogen bonds are formed, between the hydroxy groups of Tea and the acetate ions.

It is evident from these results obtained in the solid state, that the coordination spheres of the alkali and alkaline earth ions are (i) flexible and easy to distort, (ii) the coordination numbers may change depending on the conditions, and (iii) the coordination numbers may become quite large, at least up to nine. In addition, it is evident that the amino nitrogen and the hydroxy groups of the present ligands can bind to the inner coordination sphere of these metal ions, and the hydroxy groups are able to undergo hydrogen bonding.

The results described in Section 2 show that the number of coordinating hydroxy groups usually increases from Tris and Tea to Bistris. In an octahedral coordination sphere Bistris for steric reasons may occupy at the most five positions [2], while in a cubic arrangement, especially if some distortion is allowed, all six donor atoms of this ligand could coordinate. Moreover, from the X-ray analysis it is evident that all four donor atoms of Tea may easily be accomodated in a coordination sphere; as the structural Tea-unit is also part of Bistris (*cf.* Fig 1) the same may be surmised for this ligand.

Taking everything together it appears that the $M(Bistris)^{n+}$ complexes exist in aqueous solution in the form of several isomers which are in an *intra*-molecular (and hence concentration-independent) equilibrium with each other and which differ by the number of coordinated hydroxy groups. It seems

TABLE II. Extent of the Buffer-Metal Ion Interaction Expressed by the Concentration of the ML^{n+1} 1:1 Complexes for Bistris, Tea and Tris under Several Conditions. The Values are the Percentage of the Total M^{n+} Concentration Present in the Mixture as ML^{n+}, Calculated for 1:1 Conditions, *i.e.* $[M^{n+}]_{tot} = [L]_{tot} = 0.1$ *M* or 0.01 *M* (the values for this latter concentration are given in *italics*), with the Constants of Table I (for Cu^{2+} see Table III of [2]) (25 °C).

M^{n+}	Aqueous Solution (I = 1.0)						50% Dioxan (I = 0.5)		90% Dmso (I = 0.25)		
	Bistris		Tea		Tris		Bistris		Bistris	Tea	Tris
	pH 6.5	pH 7.5	pH 6.5	pH 7.5	pH 6.5	pH 7.5	pH 6.5	pH 7.5	pH 7.5	pH 7.5	pH 7.5
Li^+	1.8/ *0.2*	4.1/ *0.4*	0.09/*0.01*	0.7/*0.07*	0.09/*0.01*	0.8/*0.08*	4.8/ *0.5*	9.8/ *1.2*	15 / *2.0*	4.3/*0.5*	0.06/*0.01*
Na^+	0.5/ *0.06*	1.3/ *0.1*	0.02/*~0*	0.2/*0.02*	0.03/*~0*	0.3/*0.03*	1.7/ *0.2*	3.6/ *0.4*	13 / *1.7*	4.2/*0.5*	0.05/*~0*
K^+	≤0.5	≤1.0	≤0.02	≤0.2	≤0.02	≤0.2	≤0.9	≤2.1	9.7/ *1.2*	1.7/*0.2*	0.01/*~0*
Rb^+	≤0.5	≤1.0	≤0.02	≤0.2	≤0.02	≤0.2	≤0.9	≤2.1	8.0/ *0.9*	1.3/*0.1*	≤0.01
Mg^{2+}	6.9/ *0.8*	14 / *1.8*	0.5 /*0.05*	3.6/*0.4*	0.3 /*0.03*	2.5/*0.3*	9.9/ *1.2*	18 / *2.6*	24 / *3.9*	4.5/*0.5*	0.08/*0.01*
Ca^{2+}	68 /*31*	77 /*45*	1.6 /*0.2*	11 /*1.3*	0.3 /*0.03*	2.3/*0.2*	83 /*57*	89 /*69*	52 /*16*	8.5/*1.0*	0.04/*~0*
Sr^{2+}	38 / *8.4*	53 /*16*	0.6 /*0.07*	4.8/*0.5*	0.2 /*0.02*	1.7/*0.2*	68 /*31*	77 /*45*	60 /*23*	8.2/*1.0*	0.1 /*0.01*
Ba^{2+}	18 / *2.5*	30 / *5.4*	0.6 /*0.06*	4.6/*0.5*	0.2 /*0.02*	1.4/*0.1*	35 / *7.0*	49 /*14*	33 / *6.3*	5.3/*0.6*	0.06/*0.01*
Cu^{2+}	99 /*96*	99 /*98*	87 /*65*	95 /*86*	82 /*54*	93 /*81*					

that the one extreme is an aminoethanol-like coordination and the other a five- or six-fold coordination, depending on the steric conditions of the metal ion-coordination sphere. Additional isomers may be formed through hydrogen bonding between the hydroxy groups and coordinated water molecules; this possibility was already indicated in Section 2.2 and also follows from the described structures of the solid complexes, as well as from the series of stability in dependence on the ionic and hydrated radii (Fig. 2). The kind of isomer which dominates is most probably different for each metal ion, *e.g.* for $Ca(Bistris)^{2+}$ or $Sr(Bistris)^{2+}$ an isomer with a high degree of hydroxy group participation is expected to occur in a large percentage, whereas with $Mg(Bistris)^{2+}$ certainly a lower coordinated isomer dominates. Corresponding isomeric equilibria are also expected to occur with the $M(Tris)^{n+}$ and $M(Tea)^{n+}$ complexes. It may be added that based on a crystal structure analysis [4] of the salt $H(Tris)^+ \cdot H_2(ADP)^- \cdot 2H_2O$, it has been suggested that in solution $H(Tris)^+$ will also bind to the polar diphosphate chain of ADP through electrostatic interactions and hydrogen bonds.

General Conclusions

It should be pointed out that not only alkali and alkaline earth ions are capable of forming complexes with Tea, Tris or Bistris. In fact, several of the complexes formed with the metal ions of the second half of the 3d transition series, or with Cd^{2+} and Pb^{2+}, are considerably more stable [1, 2]. In addition, as shown previously in the systems M^{2+}/Tris/ATP and M^{2+}/Bistris/ATP, appreciable amounts of complexes containing the buffers, including mixed ligand complexes [1, 2, 57], are formed. Furthermore, the stability of several of the ternary M^{2+} complexes is quite high and it has been suggested [2] that this is due to *intra*molecular hydrogen bonding between the hydroxy groups and the phosphate oxygens of ATP^{4-}. This suggestion seems to be further substantiated by the present observations and by the X-ray structural analysis [4] of $H(Tris)^+ \cdot H_2(ADP)^- \cdot 2H_2O$.

Table II has been prepared to impart some general information on the concentration of the buffer complexes formed with alkali and alkaline earth ions. These calculations refer to $[M^{n+}]:[L]$ ratios of 1:1; an increase of the buffer concentration over that of the metal ion (or *vice versa*) will drastically increase the percentage of complexed metal ion (or buffer). It is evident that a relatively strongly coordinating metal ion like Ca^{2+} exists in the presence of certain buffers in the physiological pH range to a large extent in its complexed form, even at low reactant concentrations, while the concentration of other metal ions is influenced only at 0.1 *M* reactant concentrations. The extent of complex formation may even approach 100%, as is exemplified with Cu^{2+}*. It is evident that there are pitfalls: in each case one has to check the coordination tendency of a given metal ion towards the buffer.

An instructive example is the suggested "minimum essential medium for cultivation of mammalian cells" [22], which is 0.14 *M* in Na^+, 0.0054 *M* in K^+, 0.001 *M* in Mg^{2+} and 0.0018 *M* in Ca^{2+}; this medium is used in the presence of 0.01 *M* Bistris (in addition to other buffers) [21]. With the constants given in Table I one estimates that at pH 7 about 50% of the added Ca^{2+} exists as $Ca(Bistris)^{2+}$, while the amount of the other complexed metal ions is much lower: $Mg(Bistris)^{2+}$ ~ 1.3%, $Na(Bistris)^+$ ~ 0.1%, and $K(Bistris)^+$ < 0.1%. From the total Bistris added, about 11% exists as $M(Bistris)^{n+}$. Certainly these percentages will be altered by the presence of further ligands, like amino acids, but still this calculation demonstrates how the addition of buffers may strongly disturb the balance between freely available metal ions.

It is obvious that different metal ions are affected differently by buffers, and as pointed out, buffer-containing complexes are also easily formed in mixed ligand systems [1, 2]. In addition, certain buffer complexes may be much more stable than one would expect based on general experience [36, 47]: $Ca(Bistris)^{2+}$ is a striking example. Furthermore, the catalytic activity of coordinated metal ions may be decreased [11, 58] or enhanced [58, 59] compared to the hydrated metal ion, and a small amount of a newly appearing, highly active complex can lead to results specific for a given buffer system, but not at all for the analogous system without buffer.

There are two additional, quite general aspects which also follow from the present results. Hydroxy groups complex quite well with alkali and especially with alkaline earth ions; therefore it is to be expected that corresponding complexes are also formed with certain sugars and sugar-derivatives, *i.e.* ligand systems which are quite abundant in biological systems. The second aspect originates in the observed change in complex stability between $Ca(Bistris)^{2+}$ and $Sr(Bistris)^{2+}$ under the influence of a changing water activity (Fig. 2). This is also of interest regarding biological systems because one may expect that at the surface of a protein the activity of water is reduced, and hence the coordinating abilities of metal ions towards certain ligating groups may be favored for some metal ions and disfavored for others.

*The stability constants of the complexes between Tris, Bistris, or Tea and Mn^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, Cd^{2+}, or Pb^{2+} are listed in Table III of [2].

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