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ORIGINAL CONTRIBUTION

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Competitive binding of Na^+ and Ca^{2+} ions to teichoic acid analogues

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Abstract The competitive binding of monovalent and divalent counterions to poly(alkylene phosphate) related to bacterial teichoic acids and poly(styrenesulfonate) was studied experimentally by potentiometry with ion-selective electrodes. The binding of calcium ions and the release of sodium ions accompanying calcium ions binding in aqueous solutions of the polyelectrolytes was analysed and the mean exchange ratio Na^+/Ca^{2+} was estimated. It was found that in the process of addition of calcium ions to sodium poly(alkylene phosphate) and sodium poly(styrenesulfonate) solutions all the Ca^{2+} ions added are bound to polyions and the initially condensed Na^+ ions are released proportionally to the concentration of the added Ca^{2+} ions up to the critical concentration of the Ca^{2+} ions added. For a molar concentration ratio of calcium counterions to charged groups on the polyion up to 0.20 the exchange ratio was approximately equal to 1 or 2 for the sodium poly(alkylene phosphate)/$CaCl_2$ and sodium poly(styrenesulfonate)/$CaCl_2$ systems, respectively.

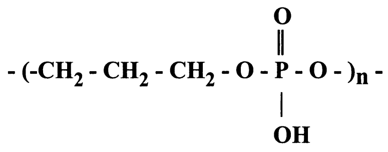
Keywords Competitive counterion binding · Polyelectrolytes · Counterion activity · Polyphosphates

Introduction

Aqueous solutions of polyelectrolytes, especially biological ones, very often contain ionic species differing in charge as counterions. In this case association of a counterion with a polyion is competitive and depends on the parameters of the system. Thus, the problem of polyelectrolytes in solutions containing counterions of different charge is important, especially for understanding the biochemical behaviour of many biopolymers, including bacterial polyelectrolytes, such as teichoic acids (TAs).

TAs are widespread among Gram-positive bacteria as a main component of their cell walls and as a part of lipoteichoic acids [1, 2, 3]. TAs of bacteria cell walls are polymeric substances consisting of a glycerol, ribitol or mannitol phosphate backbone partially substituted with various sugars or ester-linked D-alanine or both. Lipoteichoic acids are associated with the plasma membrane and they usually are composed of a poly(glycerol phosphate) chain and a glycolipid core. Besides numerous biological functions played by TAs in living organisms [1] they also play an essential role in the binding and transport of metal ions, predominantly magnesium ions, from the environment to the cell wall and plasma membrane [4, 5, 6, 7, 8].

Some fundamental structures of wall TAs and lipoteichoic acids, as well as the analogues of their polymeric backbone, were synthesized in several laboratories. One of those compounds obtained by Kałużyński et al. [9] is poly(1,3-propylene phosphate) (PPP) (Structure 1)



$$-(\text{-}CH_2-CH_2-CH_2-O-\overset{\overset{\displaystyle O}{\|}}{\underset{\underset{\displaystyle OH}{|}}{P}}-O-)_n-$$

PPP, which can be considered as a synthetic analogue of natural glycerol TAs, was investigated in our laboratory in order to solve some problems related to binding and transport of metal ions by natural TAs [10, 11, 12, 13, 14, 15, 16, 17, 18].

The competitive binding of mono- and divalent counterions in polyelectrolyte systems has been the subject of many experimental and theoretical studies. In our previous works [16, 17] we have reported experimental and theoretical studies of competitive binding of sodium and calcium ions to polyphosphate chains in solutions containing a mixture of the mentioned metal ions without additional simple salts. Now, we present the results of an analysis of the binding of calcium ions and the release of sodium ions accompanying the calcium cation binding in aqueous polyphosphate solution. Experimental data on competitive binding in the sodium poly(styrenesulfonate) (NaPSS)/$CaCl_2$ system are also included for comparison.

Experimental

PPP was prepared by polymerizing trimethylene phosphonate in CH_2Cl_2 solution using triisobutylaluminium as an initiator according to the method of Kałużyński et al. [9]. The methods of synthesis, purification and characterization of the polymer were described in previous works [14, 18]. In this work, a polyphosphate sample of degree of polymerization 25–30 was used. NaPSS of a molecular weight of about 70,000 was obtained from Aldrich Chemical Company.

Firstly, samples of PPP after a standard purification procedure [18] and NaPSS were dissolved in redistilled water and dialysed against deionized redistilled water to remove any low-molecular-weight impurities. The dialysed polymer solutions were then passed through a cation-exchange resin column converted into the acidic form (Amberlite IR 120) to obtain stock solutions of polymers in their acidic forms, HPPP and HPSS. The concentration of the stock HPPP and HPSS solutions was determined by potentiometric titration with standard 0.1 M NaOH solution. Solutions of sodium polyphosphate (NaPPP) and NaPSS were prepared by neutralizing polyacids with an equivalent amount of NaOH. All the solutions of acids and salts were stored in polyethylene bottles at 278 K.

The "activity coefficients",γ_i, of the counterions in the NaPPP/$CaCl_2$ and NaPSS/$CaCl_2$ systems were determined by electromotive force (emf) measurements of galvanic cells composed of a reference and an ion-selective electrode, as described earlier by many authors for different anionic polyelectrolytes [19, 20, 21]. The values of γ_i were calculated from the usual relationship

$$\gamma_i = a_i/c_i, \tag{1}$$

where a_i denotes the activity and c_i the concentration of the counterions, respectively.

Activity measurements of sodium and calcium ions were carried out in the same way as described in Ref. [16]. The Ca^{2+} and Na^+ activities were measured using a PHM 85 pH meter (Radiometer, Copenhagen) using a calcium-ion-selective electrode (Radiometer F 2112Ca) and a saturated calomel reference electrode (Radiometer K 401) or a solid-state ion-selective electrode (Radiometer G 502Na) and a reference electrode, respectively. The electrodes were calibrated with standard NaCl and $CaCl_2$ solutions before and after each measurement on the PPP and/or PSS solutions.

Prior to the measurements the samples were stirred and kept in a constant-temperature vessel for about 1 h to achieve thermal equilibrium, while purging with CO_2-free, water-saturated nitrogen. All the experiments were done at 298.15 ± 0.05 K. A Radiometer ABU 12 automatic burette was used to control the addition of 0.1 M $CaCl_2$ solution to 50 cm^3 NaPPP or NaPSS solution. After every addition of $CaCl_2$ the polyelectrolyte solution was stirred and purged with nitrogen for about 5 min and then activities of the metal ions were measured. Stable potentials were obtained practically 5 min after each addition of a small portion of $CaCl_2$ solution to the titration vessel; however, only a constant potential reading over the next 15-min period was regarded as a reliable emf.

Determination of fractions of free and bound counterions

Reasonable explanations of many observed properties of polyelectrolyte solutions can be obtained by assuming that some of the counterions are bound with fixed charges and the rest of counterions are free [22, 23, 24, 25, 26, 27]. However, there is no general definition of "bound" and "free" counterions. Different definitions are used by researches [16, 17].

In spite of the different definitions of "bound" counterions, it should be emphasized that many different experiments can be expressed operatively (independently of their physical counterparts at the molecular level) in terms of the apparently "free" ion concentration that is measured experimentally and the difference between the total and the measured counterion concentration, i.e. the "bound" ion concentration. Thus, the fraction of "bound" counterions (or the degree of ion binding), f_{b_i}, and the fraction of "free" counterions, f_{f_i}, where $f_{f_i} = 1 - f_{b_i}$ can be calculated from the measured activity coefficients of the counterions, γ_i, or the osmotic coefficients of the polyelectrolyte solutions, φ_p, using the simple expressions

$$f_{b_i} = \frac{A(\text{total}) - A(\text{free})}{A(\text{free})}, \tag{2}$$

$$f_{f_i} = \frac{A(\text{free})}{A(\text{total})} \tag{3}$$

and

$$\frac{A(\text{free})}{A(\text{total})} = \gamma_i \text{ or } \varphi_p, \tag{4}$$

where A denotes the concentration of the counterions.

Results and discussion

The results of the investigation of divalent cation binding and a the release of monovalent cations accompanying the divalent cation binding in NaPPP and NaPSS solutions are presented in Figs. 1 and 2.

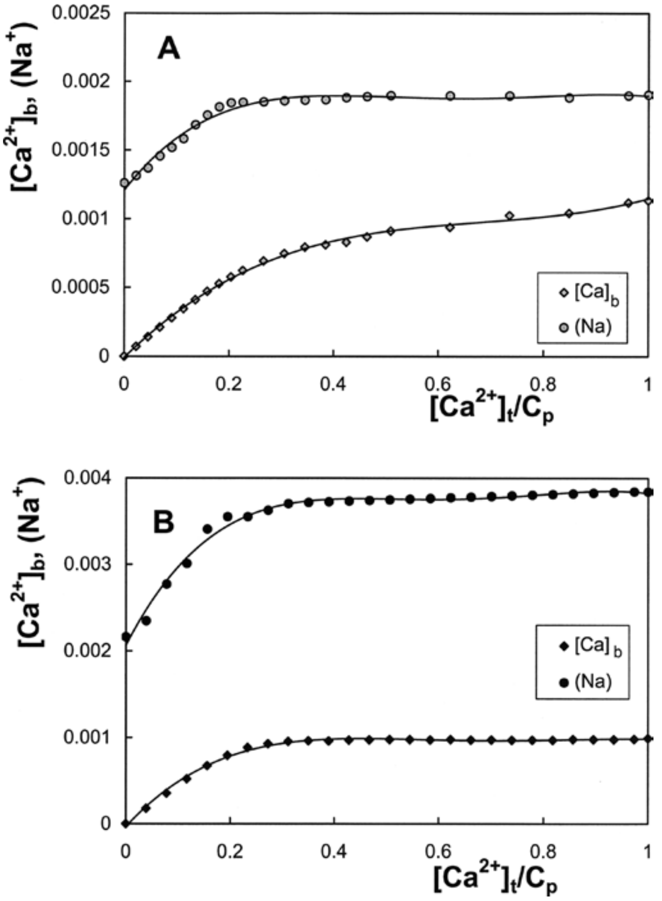


Fig. 1. Activity of sodium ions, (Na^+), and concentration of bound calcium ions, $[Ca^{2+}]_b$, in **a** sodium poly(alkylene phosphate) (*NaPPP*)/$CaCl_2$ and **b** sodium poly(styrenesulfonate) (*NaPSS*)/$CaCl_2$ systems. $[Ca^{2+}]_t$ is the concentration of calcium ions added and C_p is the concentration of the polyelectrolyte solution, $[NaPPP]=3.2\times10^{-3}$ M, $[NaPPS]=5.0\times10^{-3}$ M

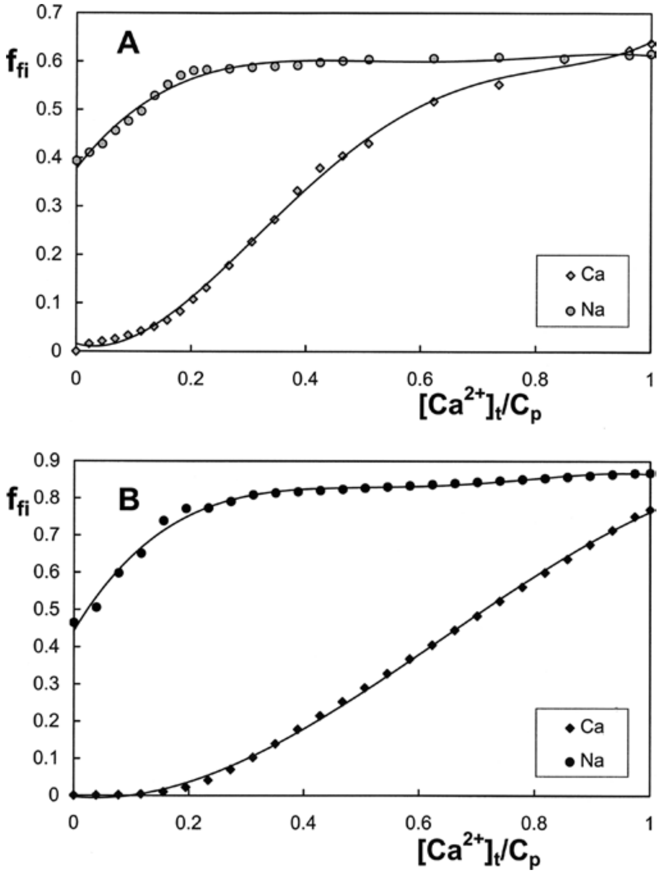


Fig. 2. Fractions of free calcium and sodium ions, f_{fi} in **a** NaPPP/$CaCl_2$ and **b** NaPSS/$CaCl_2$ systems as a function of the ratio $[Ca^{2+}]_t/C_p$; $[NaPPP]=3.2\times10^{-3}$ M, $[NaPPS]=5.0\times10^{-3}$ M

The activity of the sodium ions, (Na^+), and the concentration of bound calcium ions, $[Ca^{2+}]_b$, in NaPPP and NaPSS solutions of polyelectrolyte concentration $C_p=3.2\times10^{-3}$ and 5.0×10^{-3} M, respectively, titrated with $CaCl_2$ are presented in Fig. 1a and b. The curves $[Ca^{2+}]_b=f([Ca^{2+}]_t/C_p)$ and $(Na^+)=f([Ca^{2+}]_t/C_p)$ for all the polyphosphate solution concentrations analysed, ranging from 6.4×10^{-4} to 1.6×10^{-2} M, were similar to those presented in Fig. 1a. In the first part of the titration, i.e. for $0 \le [Ca^{2+}]_t/C_p \le 0.20$, both the concentration of the bound calcium ions as well as the activity of the sodium ions increase proportionally to the stoichiometric concentration of $CaCl_2$ added. A further addition of $CaCl_2$ beyond $[Ca^{2+}]_t/C_p=0.20$ brings only a small increase in $[Ca^{2+}]_b$ and in (Na^+). It was checked by the least-squares method that the first parts of the titration curves up to $[Ca^{2+}]_t/C_p=0.20$ are linear. The values of the correlation coefficients for linear parts of the titration curves are presented in Table 1. In this table the slopes of the linear part of the $[Ca^{2+}]_b$ versus $[Ca^{2+}]_t/C_p$ plot, B, and the (Na^+) versus $[Ca^{2+}]_t/C_p$ plot, A, for NaPPP/$CaCl_2$ and NaPSS/$CaCl_2$ systems of different C_p are also included. In the last column of this table values of the ratio $r=B/A$, which can be used as a measure of the exchange pattern [19, 28, 29, 30], are listed.

The data in Table 1 indicate that the mean Na^+/Ca^{2+} exchange ratio, r, for all the NaPPP/$CaCl_2$ systems studied, except for the case of $C_p=1.6\times10^{-4}$ M, is approximately 1.0. The result obtained for the smallest polyelectrolyte concentration seems to be due to an experimental error in measuring a rather low sodium ion activity. However, for the NaPSS/$CaCl_2$ system with $C_p=5.0\times10^{-3}$ M, r was found to be 1.80 ± 0.03, i.e. nearly 2.0, for the same range of $[Ca^{2+}]_t/C_p$.

The values of the M^+/M^{2+} exchange ratio for NaPPP/$CaCl_2$ and NaPSS/$CaCl_2$ systems obtained from potentiometric data as well as exchange ratios for other polyelectrolyte systems obtained from potentiometric and conductimetric measurements are collected in Table 2. It can be seen that for some polymer systems the

Table 1. Slope of the (Na^+)$=f([Ca^{2+}]_t/C_p)$, *B*, and $[Ca^{2+}]_b=f([Ca^{2+}]_t/C_p)$, *A*, curves and values of the Na^+/Ca^{2+} exchange ratio, *r*, for poly(1,3-propylene phosphate) (*PPP*) and poly(styrenesulfonate) (*PSS*) at $0<[Ca^{2+}]_t/C_p \leq 0.20$

Polyelectrolyte/salt system	*Cp* (moldm^{-3})	*B*	R^2 [a]	*A*	R^2 [a]	*r*
NaPPP/$CaCl_2$	6.4×10^{-4}	1.38	0.9888	0.95	0.9997	1.45 ± 0.06[b]
	3.2×10^{-3}	0.99	0.9957	0.94	0.9985	1.05 ± 0.03
	8.1×10^{-3}	0.78	0.9903	0.96	0.9997	0.81 ± 0.04
	1.6×10^{-2}	0.81	0.8906	0.95	0.9997	0.85 ± 0.06
NaPSS/$CaCl_2$	5.0×10^{-3}	1.75	0.9817	0.97	0.9995	1.80 ± 0.03

[a] Square of correlation coefficient *R* (determination coefficient)
[b] See text

Table 2. Values of the mean M^+/M^{2+} exchange ratio, *r*, for polyelectrolytes of different charge density parameter, λ, for $0<[Ca]_t/C_p \leq 0.20$. Sodium salt of poly(vinylsulfonic acid) (*NaPVS*), sodium dextran sulfate (*NaDS*), sodium salt of poly(acrylic acid) (*NaPAA*), sodium salt of poly(methacrylic acid) (*NaPMA*), degree of neutralization, α

Polyelectrolyte/salt system	λ	C_p (moldm^{-3})	*r*	Method of analysis	References
NaPPP/$CaCl_2$	0.93	3.2×10^{-3}	1.05 ± 0.05	Potentiometry with ion-selective electrodes	This work
		8.1×10^{-3}	0.81 ± 0.04		
		1.6×10^{-2}	0.85 ± 0.06		
NaPSS/$CaCl_2$	2.83	5.0×10^{-3}	1.80 ± 0.03	Potentiometry with ion-selective electrodes	This work
NaPSS/$CuSO_4$	2.83	2.0×10^{-3}	1.67	Potentiometry with ion-selective electrodes	[29]
NaPVS/$CuSO_4$	2.83	1.0×10^{-3}	1.22	Potentiometry with ion-selective electrodes	[29]
		5.0×10^{-3}	1.33		
		1.0×10^{-2}	1.36		
NaDS/$CuSO_4$	2.80	1.0×10^{-3}	2.02	Potentiometry with ion-selective electrodes	[29]
		5.0×10^{-3}	2.17		
		1.0×10^{-2}	2.09		
NaPAA/$Zn(NO_3)_2$ $\alpha=0.3$	0.76	2.5×10^{-3}	0.74 ± 0.05	Conductimetry	[33]
NaPAA/$Zn(NO_3)_2$ $\alpha=0.5$	1.42	2.5×10^{-3}	1.01 ± 0.07	Conductimetry	[33]
NaPAA/$Zn(NO_3)_2$ $\alpha=0.8$	2.27	2.5×10^{-3}	1.13 ± 0.04	Conductimetry	[33]
NaPAA/$CaCl_2$ $\alpha=1.0$	2.83	2.5×10^{-2}	1.5–1.8	Potentiometry with ion-selective electrodes	[19]
NaPAA/$MgCl_2$ $\alpha=1.0$	2.83	2.5×10^{-2}	1.5-1.8	Potentiometry with ion-selective electrodes	[19]
NaPMA/$Ca(NO_3)_2$ $\alpha=0.8$	2.27	2.5×10^{-3}	1.39 ± 0.03	Conductimetry	[34]
NaPMA/$Mg(NO_3)_2$ $\alpha=0.8$	2.27	2.5×10^{-3}	1.33 ± 0.03	Conductimetry	[34]

estimated exchange ratio is not too far from unity. However, there are also polyelectrolyte systems for which 1 mol of divalent counterions exchanges with nearly 2 mol of monovalent counterions. It is very difficult to say which factor determines the numerical value of *r*.

In the second column of Table 2 we present values of the stoichiometric charge density parameter, λ. λ is a well-known quantity that plays a prominent role in the theoretical characterization of polyelectrolytes [22, 23]. It is given by

$$\lambda = \frac{e^2}{4\pi\varepsilon_0\varepsilon k_B T b}, \tag{5}$$

where *e* is the protonic charge, ϵ is the dielectric constant of the solvent, ϵ_0 is the permittivity of a vacuum, k_B is the Boltzmann constant, *T* is the absolute temperature and *b* is the average axial charge spacing (or the length per unit charge).

The data presented in Table 2 indicate that value of *r* depends on the linear charge density parameter of the polyelectrolyte, λ, and it is generally lower for polyelectrolytes with smaller λ.

Counterion condensation theories, including the intermediate model of counterion condensation [31, 32], predict that one divalent counterion exchanges with two monovalent counterions; however, 1 mol–1 mol exchange has been also predicted. Miyamoto and Imai [28] have performed an analysis of competition between mono- and divalent counterions in polyelectrolyte solutions by applying the Poisson and Fokker–Planck equations to thin rodlike polyions. They showed that one bound divalent counterion should release one monovalent counterion.

From the calcium and sodium activity measurements in the NaPPP/$CaCl_2$ and NaPSS/$CaCl_2$ systems the fractions of free sodium counterions, $f_{f_{Na}}$, and free calcium counterions, $f_{f_{Ca}}$, were calculated using Eqs. (2), (3) and (4). It is well known that the value of γ_i can be interpreted as the fraction of free counterions [22, 23, 25, 26, 27]. In Fig. 2a and b the resulting $f_{f_{Na}}$ and $f_{f_{Ca}}$ values are plotted as a function of $[Ca^{2+}]_t/C_p$ for the NaPPP/$CaCl_2$ and NaPSS/$CaCl_2$ systems of C_p analogous to that in Fig. 1a and b. From the data presented, for both polyelectrolytes up to $[Ca^{2+}]_t/C_p=0.20$ $f_{f_{Ca}}$ is practically zero. This means that all the Ca^{2+} ions added are practically quantitatively bound to PPP or PSS polyions. Simultaneously, the fraction $f_{f_{Na}}$ increases proportionally to the stoichiometric concentration of Ca^{2+} ions added and then it is practically constant. The observed initial increase and saturation of $f_{f_{Na}}$ with increasing

$[\mathrm{Ca}^{2+}]_t/C_p$ is due to the exchange of condensed sodium ions for added calcium ions.

Our experimental results on the release of monovalent counterions by addition of divalent counterions to aqueous solutions of NaPPPs and NaPSSs are similar to those obtained earlier by other investigators for different polyelectrolytes [19, 29, 30, 33, 34]. These results seem to confirm that in the case of polyelectrolytes of lower charge densities a divalent counterion replaces less monovalent counterions than in the case of polyelectrolytes of higher charge densities.

The problem of the binding of metal ions by bacterial TAs was analysed earlier by many authors [6, 8, 35, 36, 37, 38, 39, 40, 41] using whole Gram-positive species, cell walls isolated from bacteria or pure TAs extracted from bacterial cell walls. It was found that the mode of binding of divalent metal ions, including magnesium ions, to the phosphate group of TA was different for the intact cell wall and for isolated anionic polymer in solution. The magnesium ion is divalently bound to two phosphate groups of TA contained in the cell wall, but it is univalently bound to the phosphate group and univalently to a coion in the case of TA in water solution [35, 36, 37]. Moreover, recent research on binding of metal ions by bacteria cell walls indicates that the binding of protons and divalent and trivalent metal ions onto bacterial surfaces can be effectively described in terms of surface complexation, within the framework of equilibrium thermodynamics [42, 43, 44, 45]. Fein et al. [42] and Daughney et al. [43] conclude that all surface complexes have a 1:1 stoichiometry. Further evidence in support of the 1:1 stoichiometry for the metal/bacteria surface complexes is provided by the electrophoretic mobility experiments of Collins and Stotzky [46]. These authors report that bacterial cells, which are negatively charged in the absence of metals, become positively charged in the presence of divalent metal ions. They conclude that the positive charge originates from a surface complex having one divalent metal ion coordinated to one surface functional group.

As already discussed, our results on the counterion binding in the NaPPP/$\mathrm{CaCl_2}$ system show that about one Na^+ is displaced as each Ca^{2+} becomes associated. In conclusion, we can say that our results seem to indicate that the binding mode of divalent metal ions to polyphosphates related to bacterial TAs is the same or very similar to that observed for bacterial TAs isolated from cell walls. In both cases complexes of the same or very similar structure are formed. It can be supposed that a 1:1 stoichiometry for divalent metal ion/phosphate group of PPP in water solution exists.

Conclusion

It can be concluded that potentiometry with ion-selective electrodes can be used to obtain information on the counterion distribution in polyelectrolyte/simple salt systems. The experimental results presented give an image of the composition of the counterion atmosphere around the PPP anion in solution containing both mono- and divalent counterions. The results indicate that in the process of calcium ion addition to NaPPP solution all the Ca^{2+} ions added are bound to polyphosphate ions and the initially condensed Na^+ ions are released proportionally to the concentration of the Ca^{2+} ions added, up to the critical concentration of the Ca^{2+} ions added, i.e. up to $[\mathrm{Ca}^{2+}]_t/C_p = 0.20$. The mean $\mathrm{Na}^+/\mathrm{Ca}^{2+}$ exchange ratio in the range $0 \leq [\mathrm{Ca}^{2+}]_t/C_p \leq 0.20$ is approximately 1. Comparison of results for NaPPP/$\mathrm{CaCl_2}$ and NaPSS/$\mathrm{CaCl_2}$ systems seems to confirm that the M^+/M^{2+} exchange ratio depends on the linear charge density parameter of the polyelectrolyte and it is generally lower for polyelectrolytes with a smaller value of λ. The binding mode of divalent metal ions to phosphate groups and the structure of the complexes formed by the metal ions and phosphate groups are the same or very similar, both for synthetic polyphosphates related to bacterial TAs as well as for TAs isolated from bacterial species.

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