Polish J. Chem., **75**, 1171–1181 (2001) **REVIEW ARTICLE**

Models of Biomacromolecules and Other Useful Structures Based on the Poly(alkylene phosphate) Chains*

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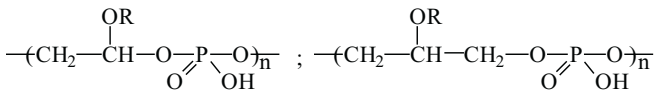
Poly(alkylene phosphate) backbones are at the basis of two important classes of biomacromolecules, nucleic and teichoic acids. Both are known to strongly bind metal cations; teichoic acids interact specifically with Ca^{2+} and Mg^{2+} cations transporting these cations in the biological milieu. This review describes the work of this laboratory directed towards synthesis of the backbones interaction with cations and some applications in nonbiological systems, although related to the ability to interact with cations. Thus, poly(alkylene phosphates) are described as liquid membranes and in the form of block copolymers as regulating crystal growth by interacting with cations rich surface. Moreover, poly(alkylene phosphates) function as strong acids: cationation of polyaniline (doping) leading to the intermolecular complex, in which poly(pentamethylene phosphate) specifically recognize the distance between the nitrogen atoms in polyaniline.

Key words: poly(alkylene phosphates), biomimicking polymers, liquid membranes, polyaniline, crystal growth modifiers

Introduction

Poly(alkylene phosphate) chains are at the bases of several classes of biomacromolecules. Besides polynucleotides there are teichoic acids-either based on glycerol, namely 1,2- or 1,3-poly(glycerol phosphates) (PGP) or on cyclic sugars, like D-ribitol [1,2].



(glycerol-1,2- and 1,3-teichoic acids)

DP_n = 25-40

(R = alanyl, sugar)

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* Dedicated to Prof. Jan Michalski on the occasion of his 80th birthday.
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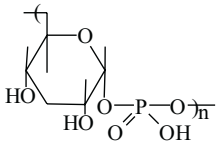
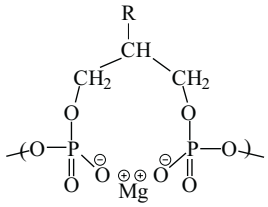


(D-ribitol-1,5-teichoic acid)

Teichoic acids (TA) are known to be responsible for the ion transport through the bacteria cell wall (wall teichoic acids) or binding Mg^{2+} on the external surface of the cytoplasmic membrane (lipoteichoic acids) [3,4]. The main physiological function of TA is to collect and preconcentrate the divalent cations, particularly magnesium ions, from the surrounding medium, and to facilitate their permeation towards the phospholipid bilayer, where their concentration is kept at the proper level. Both 1,2- and 1,3-glycerol TA could actively (*i.e.* against a gradient of concentration) transport Ca^{2+} or Mg^{2+} cations but it was not clear for a long time, which one (*i.e.* 1,2- or 1,3-) is responsible for a given cation. Indeed, the TAs have mostly been isolated from bacterial sources and could not be standardized sufficiently. Magnesium ions are associated with the cell walls in two different ways [5].

The stronger bound cations are associated with two adjacent phosphate groups in TA chains as it is shown below in Scheme 1.

Scheme 1

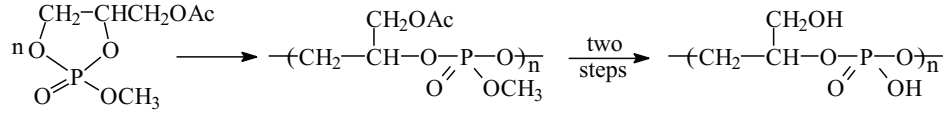


At pH = 5, $K_a = 2.7 \cdot 10^3$ (mol·L^{-1}) for Mg^{2+} and glycerol TA, whereas $K_a = 0.61 \cdot 10^3$ (mol·L^{-1}) for ribitol TA. This is because two adjacent units with rather structurally stiff sugar moiety are not allowing these units to rotate freely around the ester bond, as required for assuming conformation shown in Scheme 1. Therefore, it has become of interest to study the influence of the distance between the phosphate units on the binding efficiency in systems, where ionic interactions are important.

For this review we selected a few systems studied in our laboratory. These are: interaction of poly(alkylene phosphates) with $Na^{\oplus}$ Ca^{2+} and Mg^{2+} cations, active transport in liquid membranes, interaction of polymers containing blocks of units with monoesters of phosphoric acid with inorganic crystals or particles, and finally interaction of poly(alkylene phosphates) with polyaniline.

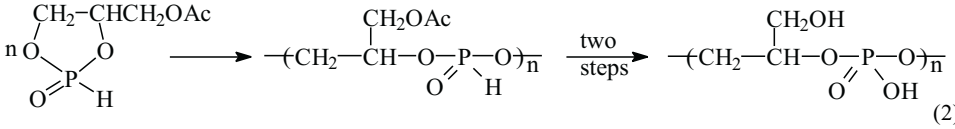
Synthesis of model macromolecules

Syntheses based on the ring-opening polymerization were described in [6,7,8,9]. Since our original interest was related to the biomacromolecules and their models, we first elaborated synthesis of macromolecules with repeating units with two or three carbon atoms between the phosphate groups. The corresponding polymers were thus prepared by ring-opening polymerization of the five- or six-membered cyclic esters of phosphoric acid or their derivatives. This strategy can be illustrated taking as an example synthesis of poly (1,2-glycerol phosphate). Five membered rings are strained (~5 kcal/mol) and polymerization proceeds to completion. The ring strain was determined in our studies of the polymerization thermodynamics [10] and earlier by Westheimer [11] in his works on hydrolysis of the five membered esters:



(1)

or



(2)

In both approaches, namely the "triesters route" and "H-phosphonate route" polymers of relatively high molar masses ($>10^4$) were prepared. The "H-phosphonate route" allowed also preparation of the high molar mass polymers with six atoms repeating units, like in the 1,3-glycerol phosphate.

Recently, we elaborated a more general method leading to poly(alkylene phosphates), not restricted to structures related to cyclic monomers with strained rings. This method is based on the well known condensation of alcohols with H-phosphonates [12,13]:

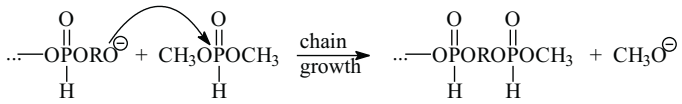
$$\text{(excess) ROH} + (\text{R'O})_2\text{P(O)H} \rightleftharpoons (\text{RO})_2\text{P(O)H} + 2\text{R'OH} \qquad (3)$$

When diol is used then polycondensation takes place and removal of an alcohol formed shifts the equilibrium to the side by the desired polymer:

$$n\,\text{HOROH} + n\,(\text{R'O})_2\text{P(O)H} \rightleftharpoons \text{HOR}\left[\text{OP(O)(H)OR}\right]_{n-1}\text{OP(O)(H)OR'} + \uparrow\text{R'OH} \qquad (4)$$

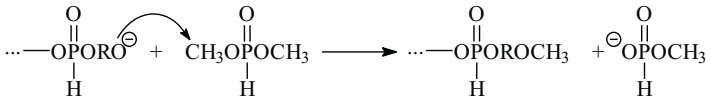
This polycondensation proceeds in the presence of the catalytic amounts of sodium or potassium alcoholate. When R′ is CH_3 or C_2H_5, as usually used in these reactions,

high molar mass products could not be prepared, because dealkylation is accompanying the major reaction of the chain growth (*e.g.* for dimethyl H-phosphonate):



(5)

Chain termination involves "wrong direction of attack" of the growing anion chain end and involving carbon atom in place of the phosphorus atom, as it is shown for the chain growth:



(6)

This dealkylation, well known in the phosphorus organic chemistry [14], was also described as a reason of molar masses limitation in the polycondensation of diols with dialkyl H-phosphonates [15]. Indeed, as shown in (6) every dealkylation would stop the growth of the polymer molecule. We have found, however, that changing the polycondensation into the polytransesterification, proceeding at higher temperature, allows preparing polymers with molar masses higher than $30 \cdot 10^3$. Mechanism of this novel process has also been discussed in our papers [12,13].

Direct polytransesterification was successfully used in preparation of polymers based on various glycols with exception of 1,3-propylene glycol and 1,4-butylene glycol; in these two instances cyclic compounds resulted. However, both ring opening polymerization and transesterification gave high molar mass poly(alkylene phosphates), starting from two methylene groups in the repeating units up to twelve [16]. Besides, poly(alkylene phosphates) were prepared from oligomeric glycols, like poly(ethylene glycol) or poly(tetramethylene glycol) [17]. Dealkylation can be fully avoided if in polycondensation diphenyl H-phosphonate is used in place of the aliphatic dialkyl H-phosphonates [18]. This process is then proceeding smoothly, even at the room temperature, since the less stable aromatic ester is replaced by a more stable aliphatic one. The equilibrium is so much shifted to the side of macromolecules, that phenol formed in this process does not have to be removed during polycondensation:

$$(C_6H_5O)_2P(O)H + nHOROH \rightleftharpoons HO[ROP(O)(H)O]_{n-1}ROP(O)(H) + C_6H_5OH \quad (7)$$

Poly(alkylene phosphates), depending on the number of atoms in repeating units, differ in their abilities to complex metal cations. This stems from difference in distances between the phosphate groups along the chains, and the arrangements differing for the even and odd number of atoms in the repeating unit of the backbone.

If we assume the zig-zag conformation, then for an uneven number of atoms in repeating unit every phosphate group along the chain is exposed to the same side of the macromolecule. For an even number of repeating units only every second is directed to the same side. This is illustrated below in Scheme 2:

Scheme 2

uneven number (*e.g.* 5):

even number (*e.g.* 6):

where J is the P atom; the other elements of the chain are thus self-explanatory.

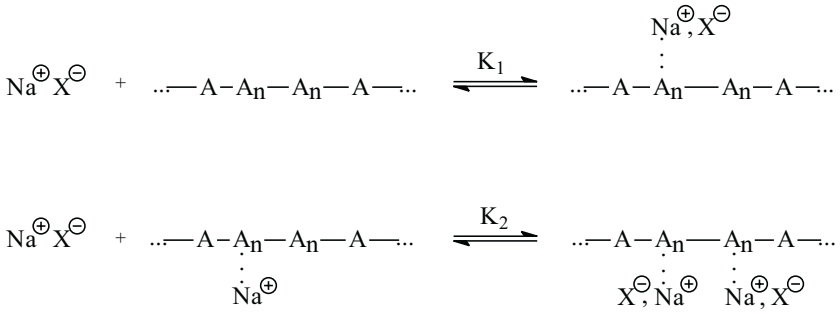
Interaction of synthetic poly(alkylene phosphates) with metal cations

There are in principle two ways these novel macromolecules can interact with cations. Either by complexation with non-ionic units, like in poly(methylethylene phosphate), thus in a similar way as poly(ethylene oxide) or crown ethers are complexing metal cations, or by acidic functions and formation of the macromolecular salts or by using both ways.

Dipol-ion interaction

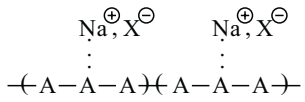
Complexation with neutral chains was studied with poly(methylethylene phosphate) and sodium cations [19]. ^{23}Na NMR was used, and the corresponding equilibrium constants were determined from the results of dynamic NMR data. Line broadening, expressed by the line width ($w_{1/2}$) allowed determination of the corresponding complexation constants K_1, K_2, K_3..., where subscripts denote the average number of repeating units responsible for complexing of one $Na^{\oplus}$ cation:

Scheme 3



It was found that the equilibrium constants K_i increase with n, until n becomes equal to 3, thus, until the structure resembles the following one:

Scheme 4



In the formulae above A = –(OP(O)(OCH_3)OCH_2CH_2)–.

The final conclusion reached was, therefore, that $K_3 > K_2 > K_1$. This is probably due to the formation of clusters, like pairs or triplets of ion-pairs.

K_3 is for $Na^{\oplus}$ close to the similar complexation constants determined for poly(ethylene oxide).

Ionic interactions

As it has been indicated in the introduction, there was a long-lasting discussion related to the responsibility of either 1,2- or 1,3-glycerol phosphate for active transport of Ca^{2+} or Mg^{2+} cations to the cell walls. The models prepared throughout our work allowed the determination of the corresponding preferences of the 1,2- and 1,3-polymers towards magnesium and calcium cations [20]. The transport phenomena were studied in a simple apparatus with three compartments, separated by solid membranes of Nafion [21]. In the first compartment (feeding phase) water solution of Ca^{2+} and Mg^{2+} of known concentrations was placed, in the middle one water solution of either polyphosphate of ethylene glycol or (in another experiment) of polyphosphate of 1,3-propylene glycol, and in the third one (stripping phase) water solution of HCl. In such a setup macromolecule of poly(alkylene phosphate) comes to the membrane, picks up mostly this cation for which it is specific, and goes to the other wall, where cations are discharged and exchanged with the membrane with $H^{\oplus}$ ions. Flame photometry is used to measure concentrations of Ca^{2+} and Mg^{2+} in the stripping phase. In this way it was shown that 1,3-polymer is more active in transporting Mg^{2+} than Ca^{2+}.

The ratio of fluxes and association constants are reflecting the corresponding preferences [20].

Table 1. Characteristics of the preference of the polyphosphates 1,2-PGP and 1,3-PGP towards magnesium ions in the competitive exchange diffusion processes.

Specification	Carrier	
	1,2-PGP	1,3-PGP
$R_1 = (S_s/S_f)_{max}$:		
Proton-coupled transport	1.65	3.70
Sodium-coupled transport	1.48	
Magnesium flux:		
$J_{Mg} \cdot 10^9/(mol \cdot cm^{-2} \cdot s^{-1})$	3.50	14.00
Calcium flux:		
$J_{Ca} \cdot 10^9/(mol \cdot cm^{-2} \cdot s^{-1})$	35.00	
Ratio of fluxes:		
$R_2 = J_{Mg}/J_{Ca}$	0.10	>> 1.00

Therefore, 1,3-PGP prefers Mg^{2+}, although Mg^{2+} cation is "smaller" than Ca^{2+} cation; Mg is 12th in the Periodic Table, whereas Ca is the 20th. This result agrees well with Scheme 2, showing that phosphate groups in 1,3-PGP are in fact closer one to another than in 1,2-PGP. The results of these studies allowed asking the next question: can other ions be separated by these or similar structures. Of practical interest for industrial electrochemists is separation of Ni^{2+} and Co^{2+} [20]. After screening of various structures the most efficient one, that was finally found, is poly[poly(ethylene glycol) phosphate], where the poly(ethylene glycol) (PEG) has an average polymerization degree n = 45. We cannot offer the exact structure of the ionic complexes formed, but apparently both dipol-ionic and ionic-ionic interactions are involved,

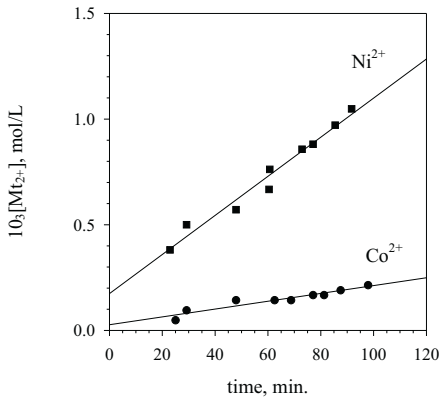


Figure 1. Competitive transport of Ni^{2+} and Co^{2+} within a liquid membrane with poly[(PEG 1000) phosphate] as a carrier.

providing this remarkable specificity in interactions, (remembering, that Co is 27th and Ni is 28th in the Periodic Table). It should be added, that neither PEG alone nor the low molar mass esters of phosphoric acid show any efficiency in separation of Ni^{2+} and Co^{2+}. In Fig. 1 the kinetics of separation of Ni^{2+} and Co^{2+} by poly[poly(ethylene glycol) phosphate] is illustrated [20].

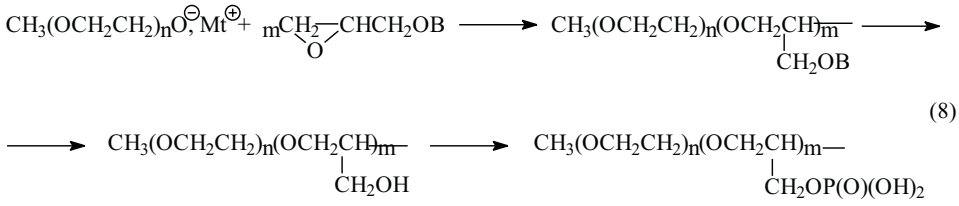
Ionic-nonionic dihydrophilic block copolymers (with phosphate units) and their interaction with inorganic particles

Strong interaction of mono- and diesters of phosphoric acid with various cations, and particularly with Ca^{2+} and Mg^{2+} cations prompted us to explore the interaction of related polymers with inorganic particles and their influence on the inorganic crystal growth.

It has already been shown, that dihydrophilic block copolymers with ionic and nonionic blocks are successfully used in modifying of the surface of inorganic particles [23]. This modification prevents aggregation of existing particles and/or influences the crystallization process, providing crystals of the shape and size both depending on the structure and amount of the polymer used [24].

The ionic blocks used till now have mostly been based on carboxylic acids; *e.g.* copolymers of acrylic or methacrylic acid [24,25].

Block copolymers with ionic block with esters of phosphoric acid have been prepared for the first time in our laboratory [26]. One of the approaches used is illustrated below:



(8)

(B = $CH(CH_3)OCH_3$)

Block copolymers were prepared with various block lengths and various degrees of phosphorylation. The efficiency of these copolymers in influencing crystallization of $CaCO_3$ was then compared with efficiencies of similar copolymers, but bearing carboxy and sulphonyl groups. In figures below, taken from our paper [26], the influence of these three different kinds of copolymers on the crystallization of $CaCO_3$ is shown. It is clearly seen, that size of crystal and their structures change dramatically, when crystallization proceeds in the presence of block copolymers. The most efficient, as it follows from comparison of the figures, are block copolymer with phosphate esters.

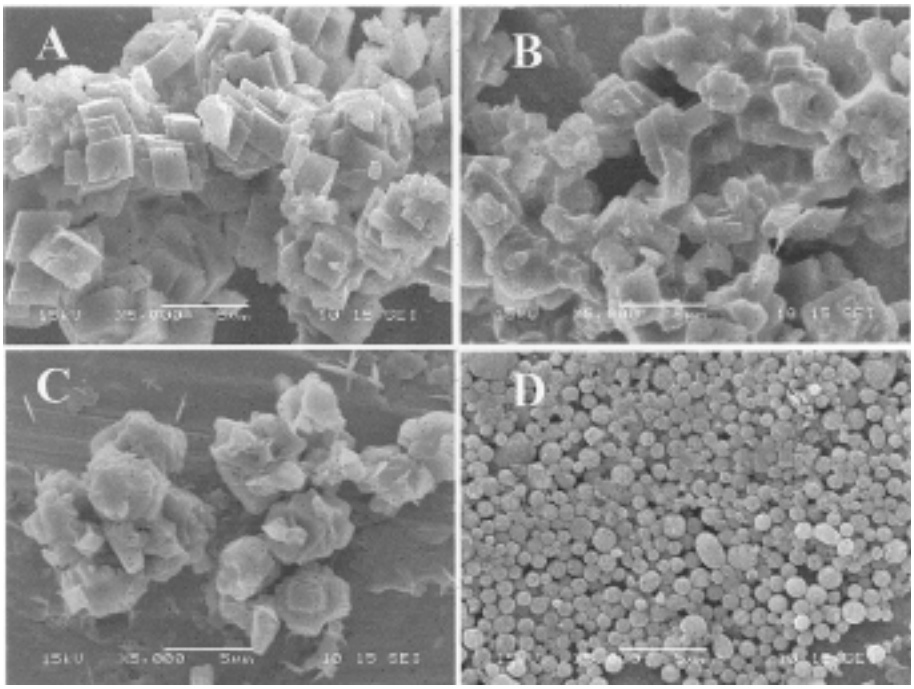
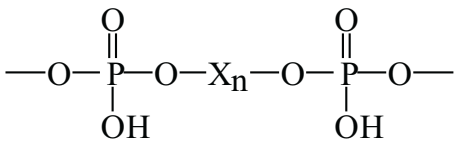


Figure 2. SEM micrographs of $CaCO_3$ crystallized: (A) – in absence of polymeric additives and in the presence of: (B) – copolymer with sulphonyl, (C) – carboxymethyl, and (D) – phosphoryl side groups. Copolymer structures explained in the text. Extend of substitution of $–CH_2OH$ groups the same for (B), (C), and (D), and equal to 50%.

The mechanism of interaction of inorganic crystals and/or particles and dihydrophilic block copolymers can be presented in the following way: on the surface of inorganic salts there are positively charged sites, due to the presence of metal cations; *e.g*. Ca^{2+}. Block copolymer having a block consisting of anions interacts with these sites and forms chemically adsorbed layer with the second, and highly hydrophilic block sticking out from the surface. The actual volume of every hydrophilic block, including the solvating molecules of water, can extend several times the actual volume of macromolecule. This is the sterically hindering layer, that prevents two particles to aggregate. The major reason, as it is belived today, is such that any close enough approach would require elimination of water molecules bound already by hydrogen bonds to a given hydrophilic block, sitting on the surface of a crystal.

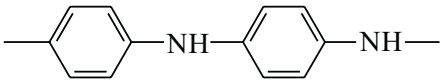
Interaction of poly(alkylene phosphates) with polyaniline

Synthetic poly(alkylene phosphates) (PAP) described in the previous sections are unique among the synthetic polyanions, because the ionic groups can be located along the chain at the chosen at will distances indicated below by X_n:

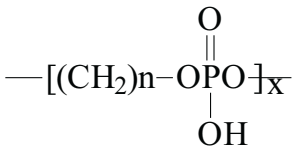


This feature makes poly(alkylene phosphates) particularly interesting for studying interaction with other polymers bearing centers rich in electrons in the chains (*e.g.* amino groups) at the fixed distances.

Since it is known, that polyanilane (PANI) has to be doped (*e.g.* protonated) in order to show increased electrical conductivity [27,28], we studied the influence of the distances between the phosphate groups in poly[(polymethylene) phosphates] (X = CH_2) on the conductivity of the corresponding complexes with polyaniline in order to establish to most efficient molecular fit, judged by the electrical conductivity of the complexes.



According to the UV-VIS spectra of the mixture of PANI with poly(alkylene phosphates) the corresponding complex is indeed formed as it may be judged from two new transitions (*ca*. 425 and 900 nm) appearing both characteristic for the protonated state of PANI [29,30]. Moreover, the presence of the isosbestic points at 460 and 770 nm proves that only two optically different phases coexist (protonated and non-protonated). The extent of protonation depends on the number of methylene units (spacer) between the phosphate groups:



Only for n = 5 the complete protonation could be achieved.

Inspection of the molecular models have shown, that when n = 5 there is a perfect fit of the phosphate groups in PAP and amino bridges in PANI [31]. This is shown below, in Fig. 3.

This observation is consistent with the result of conductivity equal to $1.5 \cdot 10^{-1}$ $S \cdot cm^{-1}$, observed for n = 5, whereas e.g. only $6 \cdot 10^{-4}$ $S \cdot cm^{-1}$ was measured for n = 12.

A thin film of PANI doped with 1 $mol \cdot dm^{-3}$ HCl solution deprotonates almost completely after its immersion into distilled water for 0.5 h, whereas PANI/PAP films have features typical of the protonated state even after 20 h of washing with water.

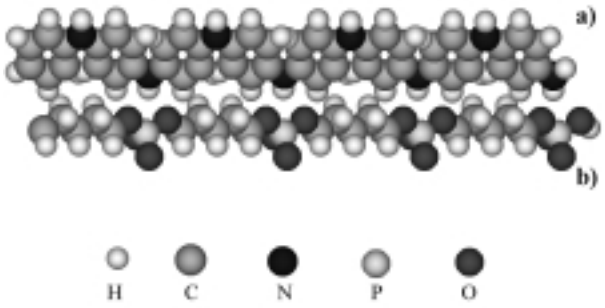


Figure 3. Computer model of polyaniline-poly(pentamethylene phosphate) complex: a) polyaniline chain, b) poly(pentamethylene phosphate) chain.

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