Self-Assembly, Antipolyelectrolyte Effect, and Nonbiofouling Properties of Polyzwitterions

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An explanation of the unique nonbiofouling properties of polyzwitterions (PZ) is proposed [in this paper, the term "polyzwitterion" is preferred to "polybetain"]. The existence of an osmotic component of the driving force of the antipolyelectrolyte effect (APE) and the parameters governing this phenomenon are quantitatively established. The correlation between this effect, which is specific of PZ only, and the PZ nonbiofouling properties is grounded.

Introduction

The two characteristic properties of polyzwitterions (PZ) creating the basis of their unique nonbiofouling properties are their low absorptivity into proteins and the preservation of the cell adhesion in the presence of PZ.[1−8] Different reasons for the observation of these properties are discussed. Attention is drawn to the structural analogy between the hydrophilic parts of phospholipids (Figure 1a) and the PZ side groups (Figure 1b,c),[9−11] both containing zwitterionic fragments forming dipoles with significant dipole moments.[12] It is namely this analogy that stimulates the synthesis and the studies of PZ as biocompatible materials. Another possible reason for the above PZ properties is the DSC-established higher value of the concentration ratio "free water/bound water" in PZ aqueous solutions and hydrogels, as compared to that of other hydrophilic polymers.[13,14] The reason for this higher value is not yet understood and its explanation is even more necessary, taking into account that hydrophilic polymers containing phosphate, sulfate, and quaternary ammonium groups do not reveal a biocompatibility similar to that of PZ. In the present paper, an explanation of these two PZ properties is proposed. It is based on the specific structural organization resulting from the specific dipole−dipole interaction between the side groups of the PZ macromolecules.

A theoretical (all-atom molecular dynamics) analysis of the dipole−dipole interaction[12,15,16] proves that the formation of fluctuational self-assemblies of oppositely oriented dipoles (Figure 2) is energetically most favorable. In this way, a new type of a specific (dipole−dipole) process of self-assembly is proposed, which differs from the conventional hydrophobic, ionic, and hydrogen-bond formation processes. The self-assemblies are specific nodes of the dynamically changing physical network and, for this reason, the PZ macromolecules are globulized. The formation of a zwitterionic phase containing these self-assemblies (nodes) is established[17−20] and a diphase structural organization of PZ can be deduced. These concepts are not used so far for the explanation of the PZ nonbiofouling properties.

Results and Discussion

Relation between the Antipolyelectrolyte Effect of PZ and Their Diphase Structure. The essence of the antipolyelectrolyte effect (APE) consists of the increase of the viscosity of a PZ solution by the addition of a low-molecular salt (LMS). It is opposite to the polyelectrolyte effect (PE) which consists of a decrease of the viscosity of a polyelectrolyte solution by the

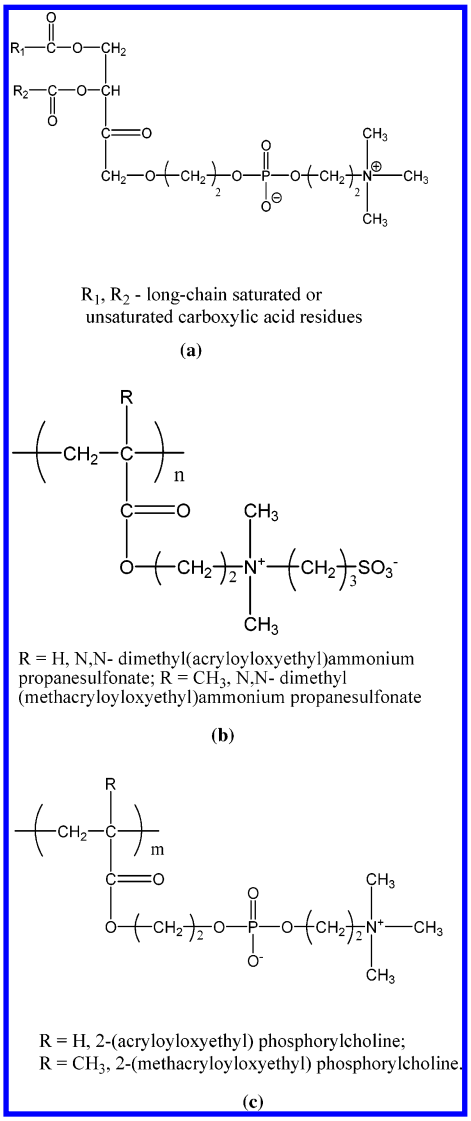

Figure 1. Structural analogy between the zwitterionic fragments of phospholipids (a) and the synthetic PZ (b) and (c).

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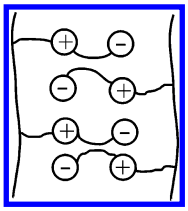


Figure 2. Dipole−dipole self-assemblies of oppositely oriented dipoles − zwitterionic side groups of PZ macromolecules.

addition of LMS. The explanation of PE is the shielding by low-molecular ions of the electrostatic repulsion between the point charges (of the same charge) along the polyelectrolyte macromolecule, and the globulization of this preliminarily swollen (due to electrostatic repulsion) macromolecule. APE is explained in an analogous manner. In this case, the shielding effect of the low-molecular ions results in the swelling of the already globulized PZ macromolecule. The reason for this globulization before the addition of LMS is usually unspecified. It is assumed that as far as PZ are polyampholytes in an isoelectric state the globulization of PZ macromolecules in the absence of salts is due to the same reasons that give rise to the shrinking of polyampholyte macromolecules upon equalization of the densities of their two electric charges (transition into the isoelectric state). A considerable difference between these two cases is ignored. In polyampholytes, the macromolecular shrinkage is a result of the interaction between opposite point charges, whereas in PZ the globulization may be the result of an interaction just between dipoles. In the latter case, in addition to the equality of the densities of the two charges, a specific orientation of the dipoles is also required. The vectorial nature of the dipole−dipole interactions predetermines the formation of self-assemblies of oppositely oriented dipoles (Figure 2) playing the role of physical nodes upon cross-linking-globulization of the PZ macromolecules. In this way, it can be assumed that the reason for PZ globulization in the absence of LMS, and hence for APE, is the formation of dipole−dipole self-assemblies (Figure 2). Consequently, this process is responsible both for APE and for the diphase structural organization of PZ. It turns out, however, that the common initial cause for APE and the diphase nature of PZ is not solely their interrelation. The PZ diphase nature gives rise to an additional osmotic component of the driving force of APE, which is usually related to the shielding effect of the low-molecular ions on the electrostatic interaction between the macromolecular ions.

Osmotic Component of APE. The diphase PZ structural organization can be represented schematically as a two-chamber cell, its two chambers being separated by a membrane which is permeable to the low-molecular ions only (Figure 3). Let LMS be a symmetrical 1−1 (CA) electrolyte, which is initially dissolved in the aqueous phase (W). The subscripts "0" and "e" denote the concentrations of low-molecular ions at the moment of addition of LMS into the aqueous phase (Figure 3A) and at the establishment of the equilibrium distribution of these ions between the two phases (Figure 3B). The superscripts (PZ) and (W) denote two phases: the polyzwitterionic (PZ) and the aqueous (W) one.

At the initial moment

$$C_{C^+,0}^{(W)} = C_{A^-,0}^{(W)} = C_{S,0}^{(W)} \tag{1}$$

In the above expression, $C_{S,0}^{(W)}$ denotes the LMS concentration at the moment of its addition into the aqueous phase. From the

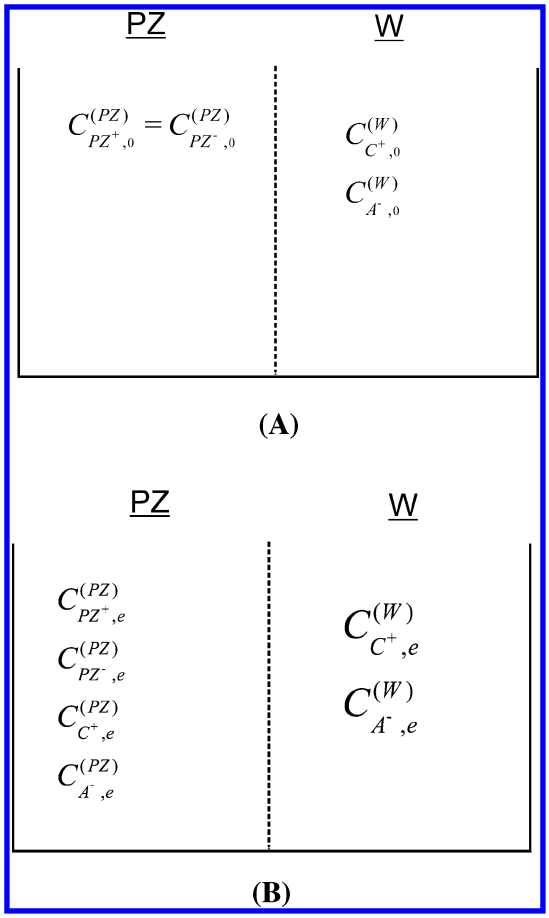


Figure 3. Schematic representation of the diphase PZ structural organization using a two-chamber cell divided by a membrane that is permeable to low-molecular ions only. At the initial moment (A) the left chamber (PZ) is occupied by dipole−dipole self-assemblies only, while the right chamber (W) contains an aqueous solution of low-molecular ions C^+ and A^-. At equilibrium (B), part of C^+ and A^- penetrate the membrane and reach the PZ chamber, the latter being originally occupied by self-assemblies only.

thermodynamic condition for uniform distribution of C^+ and A^- between the two phases

$$\mu_{C^+,e}^{(PZ)} + \mu_{A^-,e}^{(PZ)} = \mu_{C^+,e}^{(W)} + \mu_{A^-,e}^{(W)} \tag{2}$$

it follows that

$$C_{C^+,e}^{(PZ)} C_{A^-,e}^{(PZ)} = C_{C^+,e}^{(W)} C_{A^-,e}^{(W)} \tag{3}$$

In this case, an equilibrium is established after the transfer of low-molecular ions from the aqueous phase into the polyzwitterionic one, which means that their concentration in the aqueous phase is decreased. This decrease could not change the water phase volume, $V^{(W)}$, if the LMS transport is not accompanied by water transport from the W to the PZ phase as well. The increase of the concentrations C^+ and A^- in the polyzwitterionic phase will depend on the volume ratio between the two phases, $V_e^{(PZ)}$ and $V_e^{(W)}$. Let

$$\frac{V_e^{(W)}}{V_e^{(PZ)}} = k \tag{4}$$

For dilute PZ solutions, $V_e^{(PZ)} \ll V_e^{(W)}$ and for this reason $k \gg 1$. This volume ratio defines the increase of the equilibrium

concentrations $C_{C^+,e}^{(PZ)+}$ and $C_{A^-,e}^{(PZ)}$ to a value kX, X being the LMS concentration decrease in W. After introduction of this value in eq 3, the condition for equilibrium takes the form

$$(C_{S,0}^{(W)} - X)^2 = (kX)^2 \tag{5}$$

and for the decrease X one obtains

$$X = \frac{C_{S,0}^{(W)}}{1+k} \tag{6}$$

Since by definition after substitution of X in these equations,

$$C_{C^+,e}^{(W)} = C_{C^+,0}^{(W)} - X = C_{S,0}^{(W)} - X \tag{7}$$

$$C_{A^-,e}^{(W)} = C_{A^-,0}^{(W)} - X = C_{S,0}^{(W)} - X \tag{8}$$

for the equilibrium concentrations of C^+ and A^- in the aqueous phase one obtains

$$C_{C^+,e}^{(W)} = C_{A^-,e}^{(W)} = \frac{k}{1+k} C_{S,0}^{(W)} \tag{9}$$

The concentrations of low-molecular ions in the polyzwitterionic phase are kX

$$C_{C^+,e}^{(PZ)} = C_{A^-,e}^{(PZ)} = kX = \frac{k}{1+k} C_{S,0}^{(W)} \tag{10}$$

and, at equilibrium, they are the same as those in the aqueous phase. The expressions show that the deviation of these concentrations from their initial value ($C_{S,0}^{(W)}$) is the more significant, the smaller the volume ratio k. Equation 10 shows the lack of Donnan effect in PZ aqueous solutions, which should be expected since PZ are polyampholytes in the isoelectric state and, in the PZ phase, the equality of the concentrations of the two ions ($C_{PZ^-}^{(PZ)} = C_{PZ^+}^{(PZ)}$) is always observed.

The expression for X (eq 6) also allows one to derive of the interesting relationship for the volume ratio of the PZ phase at the initial moment ($V_0^{(PZ)}$) and at equilibrium ($V_e^{(PZ)}$). To this purpose, the material balance is used of the low-molecular salt distributed between the two phases at equilibrium

$$V_e^{(PZ)} kX + V_e^{(W)}(C_{S,0}^{(W)} - X) = V_0^{(W)} C_{S,0}^{(W)} \tag{11}$$

where $V_0^{(W)}$ is the initial volume of the aqueous phase. After substitution of the expression for X (eq 6), one obtains

$$\frac{V_e^{(PZ)}}{V_0^{(PZ)}} = \frac{V_0^{(W)}}{V_0^{(PZ)}}\left(\frac{1+k}{k} - \frac{V_e^{(W)}}{V_0^{(W)}}\right) \tag{12}$$

The difference in brackets is a positive magnitude since $(1 + k)/k > 1$ and $(V_e^{(W)}/V_0^{(W)}) < 1$. Since for dilute PZ solutions $(V_0^{(W)}/V_0^{(PZ)}) \gg 1$, an important inequality can be written

$$\frac{V_e^{(PZ)}}{V_0^{(PZ)}} \gg 1 \tag{13}$$

i.e., at the establishment of an equilibrium distribution of LMS between the two phases, the volume of the PZ phase ($V_e^{(PZ)}$) becomes much greater than the initial one ($V_0^{(PZ)}$). In practice, this means a substantial swelling of the PZ macromolecules upon the addition of LMS, which is the essence of APE. It should be stressed, however, that in the derivation of inequality (13) the use of the shielding effect of the low-molecular ions penetrating the PZ phase is not required. It follows that, in addition to the generally accepted reason for APE stated above, the diphase PZ structural organization proposes yet another, as seen by the derivation of inequality (13), osmotic driving force for this effect. This osmotic component is characteristic solely of PZ, since polyzwitterions are the only substances where a phase of dipole−dipole self-assemblies (Figure 2) can be formed in the absence of LMS. Both the electrostatic and the osmotic component can contribute to the APE driving force. Since both components act in the same direction, their separate identification requires the development of special techniques.

The discussed transport of low-molecular ions from the aqueous to the zwitterionic phase can be related to the widely used salt-philicity of PZ.[21−26] From eqs 9 and 10, it follows that

$$C_{C^+,e}^{(W)} = C_{C^+,e}^{(PZ)} = C_{A^-,e}^{(W)} = C_{A^-,e}^{(PZ)} \tag{14}$$

which means that at equilibrium the concentrations of the two low-molecular ions (C^+ and A^-) are the same in the aqueous and in the zwitterionic phase. Since this holds for any interphase exchange, a question arises concerning the essence of the term "PZ salt-philicity". To answer this question, one should take into account that, in addition to equalities (14), in the case of PZ, inequality (13) is also valid, and as stated above, it proves the substantial swelling of the PZ phase. This means, however, that the amount of LMS in the PZ phase is greater than that in the absence of swelling of this phase. It is namely the increase of the amount of LMS absorbed in the PZ phase as a result of swelling that represents the essence of PZ salt-philicity.

Relation between the Unique Biocompatibility of PZ and Their Ability to Swell in the Presence of LMS. It was already shown that inequality (13) and, more particularly, its derivation propose a new approach to the interpretation of APE, whereas eq 12 allows the determination of the effective governing parameters for the control of this effect. This equation shows that the relative change in the volume of the PZ phase ($V_e^{(PZ)}/V_0^{(PZ)}$) upon addition of LMS is the more significant, the greater the initial volume ratio between the two phases ($V_0^{(W)}/V_0^{(PZ)}$), the latter being much higher than unit in the case of dilute polymeric solutions. For this reason, the value of this ratio is the first and probably the main governing parameter for the control of the swelling of the PZ phase. However, the correctness of this statement requires a more detailed analysis of the relation between this parameter and the two terms in brackets in eq 12 ($(1 + k)/k$ and $V_e^{(W)}/V_0^{(W)}$). To this purpose, use is required of the volume balance upon swelling of the PZ phase. Let V_{tot} be the total volume of the system composed of PZ and W phases. Since V_{tot} does not change upon swelling, then

$$V_{tot} = V_0^{(W)} + V_0^{(PZ)} = V_e^{(W)} + V_e^{(PZ)} \tag{15}$$

If ΔV is the increase of the PZ phase at the establishment of equilibrium after the addition of LMS, i.e.

$$V_e^{(PZ)} = V_0^{(PZ)} + \Delta V \tag{16}$$

it follows, from the combination of the last two equations, that

$$V_e^{(W)} = V_0^{(W)} - \Delta V \tag{17}$$

Equations 16 and 17 are interesting in that they demonstrate the obvious conclusion (at V_{tot} = const) that the volume increase of the PZ phase is the same as the volume decrease of the aqueous phase. This imposes some dynamics in the position of the membrane depicted in Figure 3. Upon the transition from Figure 3A to Figure 3B (a transition from the initial to the equilibrium state), the membrane should shift to the right so that the PZ volume increases by ΔV, whereas that of the aqueous phase decreases by ΔV.

First, the dependence of the first term in the brackets of eq 12 on the ratio ($V_0^{(W)}/V_0^{(PZ)}$) can be analyzed. From the definition of k (eqs 4, 16, and 17), it follows that

$$k = \frac{V_e^{(W)}}{V_e^{(PZ)}} = \frac{\dfrac{V_0^{(W)} - \Delta V}{V_0^{(PZ)}}}{1 + \dfrac{\Delta V}{V_0^{(PZ)}}} \tag{18}$$

If one introduces the equilibrium volume ratio of the swelling

$$SR = \frac{\Delta V}{V_0^{(PZ)}} \tag{19}$$

then

$$k = \frac{\dfrac{V_0^{(W)}}{V_0^{(PZ)}} - SR}{1 + SR} \tag{20}$$

In the general case, SR is independent of the initial volume of the PZ phase and can be assumed to be constant. Then, the above expression shows that the higher the discussed volume ratio ($V_0^{(W)}/V_0^{(PZ)}$), the higher the k value. It follows that by the increase of this volume ratio, the first term in the brackets of eq 12 becomes closer to its unit limit. However, since by definition $k \gg 1$, the ratio $(1 + k)/k$ is always quite close to unit and the additional increase of k leads to an insignificant decrease of the ratio $(1 + k)/k$.

Using eq 17, the second term in the brackets of eq 12 can be represented in the following manner

$$\frac{V_e^{(W)}}{V_0^{(W)}} = 1 - \frac{\Delta V}{V_0^{(W)}} \tag{21}$$

However, from eq 19 it follows that

$$\Delta V = (SR)V_0^{(PZ)} \tag{22}$$

and

$$\frac{V_e^{(W)}}{V_0^{(W)}} = 1 - \frac{SR}{V_0^{(W)}/V_0^{(PZ)}} \tag{23}$$

The last equality clearly shows that at constant SR, the ratio ($V_e^{(W)}/V_0^{(W)}$) should increase with the rise of ($V_0^{(W)}/V_0^{(PZ)}$). From the dependences of the two terms in the brackets of eq 12 on the multiplier ($V_0^{(W)}/V_0^{(PZ)}$), it is seen that, by the increase of this multiplier, the difference in the brackets diminishes. However, at very high values of the initial volume ratio of the two phases ($V_0^{(W)}/V_0^{(PZ)}$), the changes in the bracketed part of eq 12 are very small. For this reason, the value of the initial volume ratio ($V_0^{(W)}/V_0^{(PZ)}$) is the most important factor governing the value of the relative change in the volume of the PZ phase upon equilibrium in the presence of LMS. The performed analysis of eq 12 is valuable because it depicts the governing parameters for the control of the swelling of the PZ phase and shows the complex dependence of the value of this swelling on the initial volume ratio of the two phases ($V_0^{(W)}/V_0^{(PZ)}$). For this reason, expression (12) bears much more information than the other possible expression describing the same swelling

$$\frac{V_e^{(PZ)}}{V_0^{(PZ)}} = 1 + SR \tag{24}$$

which is obtained from eqs 16 and 22.

There are at least two reasons determining the importance of the analysis of the PZ phase swelling upon addition of LMS: (i) all cellular and extracellular processes take place in aqueous/salt solutions, i.e., PZ are in the swollen state, and (ii) in the presence of LMS, only PZ and the polyampholytes in the isoelectric state undergo swelling. All other types of water-soluble polymers (nonionogenic polymers, polyelectrolytes and polyampholytes in the nonisoelectric state) aggregate and often form a separate phase by the addition of LMS (Scheme 1). It is amazing that this specificity of the PZ aqueous solutions and hydrogels has not been so far related to the unique PZ biocompatibility. A possible reason in this respect could be the lack of clarity concerning the osmotic component of the APE driving force, which is established in the present report.

Now, after the unambiguous demonstration of the existence of this component and the proved disintegrationof the dipole−dipole self-assemblies, the correlation between the nonbiofouling properties and the swelling ability of PZ by the addition of LMS seems reasonable and acceptable. This disintegration and the swelling of the PZ macromolecules suggest the reason for the increased "free/bound" water ratio in PZ solutions established by DSC.[13,14] This reason was assumed to create the basis of the PZ nonbiofouling properties. Indeed, upon this destruction, the individual zwitterionic groups are the only elecroneutral entities in the swollen PZ phase (Figure 4).

It is seen in Figure 4 that the counterion atmospheres of these groups form the hydrophilic shell around the hydrophobic core of each PZ macromolecule, composed of a backbone and segments between the two zwitterionic charges. Inequality (13) shows that namely these strongly swollen hydrophilic shells are responsible for the higher content of "free" water detected in PZ solutions and hydrogels and assumed to be the reason for the excellent PZ nonbiofouling properties.[27,28]

However, there is one more possibility, not mentioned in the literature and quite essential for the preservation of the native state of biopolymers and cell membranes, as well as for the interaction between the latter and swollen PZ macromolecules (Figure 4). It concerns the interaction between the cores (Figure 4) and the hydrophobic areas produced at any perturbation of the native state of protein globules or cell membranes.[9,11,13,29−31] The binding between the hydrophilic and the hydrophobic parts of the PZ macromolecules is of particular importance for this interaction. When the hydrophobic cores interact with the hydrophobic fragment of the protein globule or cell membrane, the hydrophilic shell covers the produced hydrophobic "pocket", and its surface also becomes hydrophilic (Figure 5).

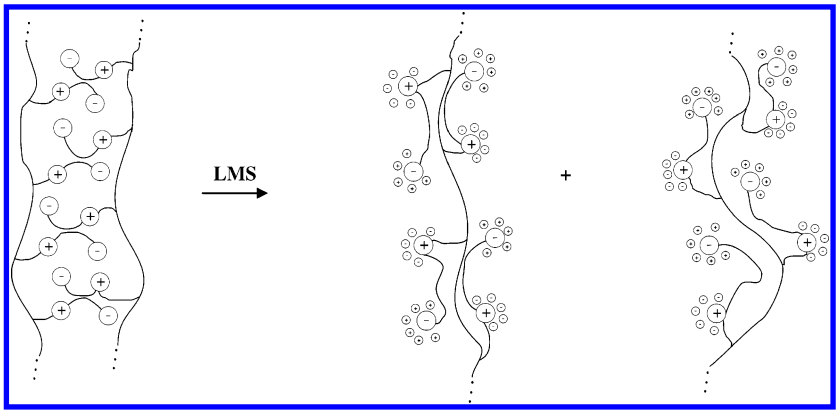
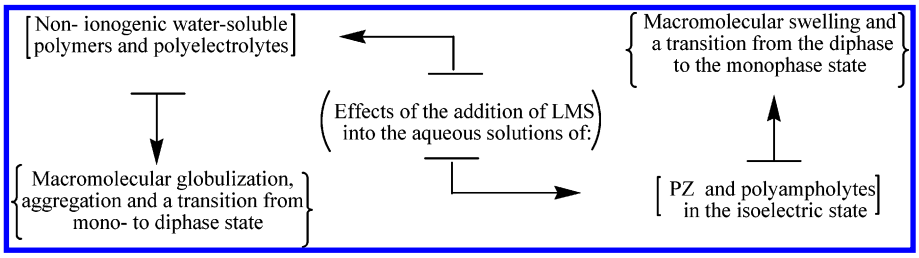


Figure 4. Disintegration of the fluctuational dipole−dipole self-assemblies by the addition of LMS into PZ aqueous solutions and formation of individual zwitterionic groups with a counterion atmosphere around each coion, strongly swollen hydrophilic shell and hydrophobic core.

Scheme 1. Comparison of the Behavior of a PZ Aqueous Solution upon Addition of LMS and that of Other Water-Soluble Polymers.



Namely, this transformation hampers or prevents the further undesired conformational transitions in the protein macromolecules and the hydrophobic interaction both between protein globules and cell membranes. It allows a new approach to the stabilization of the native conformation. In fact, the stabilization of enzymes by PZ is already proven.[32] Moreover, it has been demonstrated that the nondetergent zwitterions are protein-folding helpers,[33−35] similarly to chaperones.[36] It is interesting to check the application of this PZ action against the protein domain swapping as well.[37−39] Recently, the use of PZ as an acid phosphatase folding helper was shown.[40] The detailed analysis of this finding is not performed so far, and will be the object of a future study. The absence of (i) zwitterionic groups, (ii) a self-assembly stimulated by dipole−dipole interactions, and (iii) an APE effect expressed in strongly swollen amphiphilic PZ macromolecules in the other classes of water-soluble polymers as well as the aggregation of the latter in the presence of LMS (Scheme 1) excludes the creation of such favorable conditions for the protein macromolecules and cell membranes.

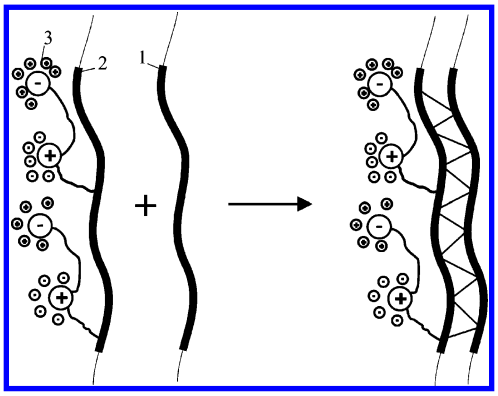


Figure 5. Schematic representation of the interaction between the core of the amphiphilic PZ macromolecule and the hydrophobic area on the protein globule surface or cell membrane, resulting in the transformation of this area into a hydrophilic one: (1) hydrophobic area on the protein globule surface; (2) a fragment from the core of the PZ macromolecule; (3) hydrophilic shell of PZ macromolecule.

The transformation of the hydrophobic surface of the latter into a hydrophilic one upon interaction with swollen PZ macromolecules is also excluded in other water-soluble polymers. The protein macromolecules and the cell walls in the PZ aqueous solutions and hydrogels are usually in contact precisely with the swollen hydrophilic shells of the PZ macromolecules shown in Figure 4. Due to their high content of "free" water and the suitable concentration of low-molecular ions, these contacts create favorable conditions for the preservation of native conformational state of proteins, other biopolymers, and cell membranes. These conditions exclude the conformational transformations required for the protein adsorption and cell adhesion.[29,30] Proteins undergo sorption on conventional polymers and the nonfouling properties of the latter are either nonexistent or negligible, as compared to those of PZ. This holds also for the common polyelectrolytes where a counterion atmosphere is also formed, but the presence of Donnan and polyelectrolyte effects decreases considerably the swelling of the external hydrophilic shells bearing "free" water.

Conclusion

An original theory is proposed for the explanation of the unique PZ nonbiofouling properties. It is based on the diphase structural organization of the PZ aqueous solutions and hydrogels in the absence of LMS. This structural organization is generated by the formation of electroneutral dipole−dipole self-assemblies of oppositely oriented dipoles. The existence of an osmotic component of the APE driving force, which is responsible for the PZ swelling by the addition of LMS is quantitatively shown. An analytical expression is derived, allowing one to outline the effective governing parameters for the control of this swelling. This APE, which is specific for PZ only, results in amphiphilic PZ macromolecules with strongly swollen hydrophilic shells and hydrophobic cores. It is stressed that the swelled hydrophilic shells provide a higher content of "free" water and favorable conditions for the native conformational state of proteins and cell membranes, whereas the

hydrophobic cores interact with the hydrophobic areas on the protein globule or cell membrane surfaces and transform them into hydrophilic ones. In this way, the PZ macromolecule hampers or prevents the protein fouling and cell adhesion, and the correlation between these peculiarities of PZ and their unique nonbiofouling properties is discussed.

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