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Polyelectrolytes at the endothelial cell surface

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Abstract

It is recalled that the tension in a stretched polyelectrolyte chain mechanically compensates both the coulomb interaction and the hydrostatic pressure increase around the chain in a compromise which minimises the free energy and keeps water chemical potential constant throughout. Stretching strongly favors parallel cylinder nematic order in polyelectrolyte brushes on a surface or in the slit between two surfaces when the polyelectrolyte chains function as bridges. Strong, stiffly stretched chains result when the molarity of the fixed charge distribution is larger than the molarity of the neutral salt solution with which the brushes are in equilibrium. The relevance of these two systems to the endothelial cells which cover the walls of blood vessels is discussed.

Keywords: Polyelectrolyte brushes; Mechanical balance; Interendothelial cleft; Glycocalyx

1. Introduction

Concentration differences in a solution at uniform temperature can be compensated in thermodynamic equilibrium by suitable differences in hydrostatic pressure. To maintain a concentration difference or concentration gradient in equilibrium thus presents no problem in principle, if a device is available, which mechanically can create and permanently maintain a stress pattern which statically exactly compensates the difference or gradient in hydrostatic pressure [1].

A membrane, a swollen gel network any similar such macroscopically "solid" device, fulfills the requirement. Even a single macromolecule within its domain [1].

When some of the solutes in an aqueous solution are electrically charged and also the permanent stress pattern creating device bears a charge, it must mechanically balance not only the hydrostatic pressure gradient but also the coulombic charge/charge interactions which derive from the "fixed" charge distribution.

Let us consider the simplest example, a charged, linear chain, flexible polymer molecule, a polyelectrolyte. The charged groups ionize and the counter ions are released. If they stay close to the groups of compensating charge on the polyelectrolyte they produce a large concentration gradient of counter ions around the polyelectrolyte. This affects solvent chemical potential and has to be compensated by a pressure increase around the polymer chain and the polyelectrolyte expands in order to provide the mechanically balancing tension If, on the other hand, the counter ions try to distribute uniformly throughout the solution the effect on solvent chemical potential is largely cancelled, but a very large electrical potential gradient is set up around the polyion which has to be compensated by polyion expansion. The molecule uncoils and the free energy rises in either case. The optimal situation is a compromise between the

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two extremes envisaged. Equilibrium is determined by a free energy minimum and the requirement that the solvent (i.e. the water) chemical potential be uniform. In the final outcome, as a rule, the electrical potential difference tends to be kept low and most of the compensation is via a relatively large counter-ion concentration gradient and the hydrostatic pressure gradient which it induces.

Although the principle of mechanical compensation by a permanently maintained stress pattern should by now be well understood, the macromolecular swelling induced complementary hydrostatic pressure gradient is generally interpreted as a direct effect (the "osmotic pressure") of the concentration gradient. Forgotten is the fact that the osmotic pressure is *not* a pressure but the effect of a finite solute concentration on the solvent chemical potential expressed in terms of pressure units. This may seem to be merely a mental short cut but it leads to untold confusion and misinterpretations. What is forgotten, in particular, is that the osmotic "pressure" is only compensated by a real hydrostatic pressure in a suitable system, i.e. if such a pressure can be mechanically generated (or is forced to be created) by some formed element of the system, for example, by the expansion of a polymer coil, or the swelling of a polymer gel network, or by the flexing of a semi-permeable membrane.

In the polyelectrolyte case the fixed charge distribution merely introduces another element into the discussion, since the electrical potential gradient gives rise, due to coulomb interactions, to other mechanical effects. As already mentioned a compromise is found in the polyelectrolyte (polyelectrolyte gel) case, whose nature is understood. Conceptually, therefore, we have here a solved problem, but in practice it is extremely difficult to model since, in the case of coulomb interactions, even slight simplifications of the exact charge distribution can very sensitively affect the result.

The theoretical approaches via intuitive model assumptions have generally involved shielded potentials of the Debye–Hückel type and a fixed charge uniformly smeared out over the length of the polyelectrolyte chain. This results in a counter-ion atmosphere in the form of a cylindrical sheath surrounding the polyelectrolyte chain. Chain expansion is taken to be sufficiently large so that close approach between remote points on the polymer chain is rare, in dilute solution, and in semi-dilute solution is prevented by the tendency of the chains to organise in some sort of nematic local order. Each polymer chain in a semi-dilute system is considered surrounded by two cylindrical sheaths, an inner, the ion atmosphere, and an outer, whose contents are electroneutral. Since the entire system is, therefore, assumed to be composed of essentially parallel, equally spaced polymer chains [2], the outer sheath encompasses that part of the entire solution space assigned on the average to each chain. The outer sheaths of neighboring chains are, therefore, in touch and the electrical potential gradient is zero over these surfaces. This inherently reasonable approach gives rise to an analytically tractable model [2], but in the absence of an exact solution with which to compare the model may or may not pay off in any particular case.

2. Polyelectrolyte brushes

Probably the most reasonable case to which to apply the model of local nematic order is the so-called polyelectrolyte-brush case. The system here involves a set of end-grafted regularly spaced polyelectrolyte chains in contact with a half-space filled with an aqueous solution but bounded on one side by the plane interface to which the chains are grafted. The distance between neighboring grafted chains is generally taken to be much smaller than the Flory radius of the uncharged polymer. One can also envisage a slit system where the slit is filled with an aqueous medium and the polyelectrolyte chains are grafted one end on one face of the slit and the other end grafted to a point on the other side of the slit exactly opposite the first end. In this case the distance between neighboring chains need not be restricted. In both systems a cylinder-like geometry is sterically imposed on the polyelectrolyte chains by the end grafts.

It is interesting that an example of each of these two systems arises in the case of the pavement of cells, the endothelial cell cover, which

constitutes the innermost layer of the blood vessel wall [3,4]. Our discussion of these two cases will be qualitative since important structural details are still not known. We will merely analyse the essential functions these polyelectrolyte brushes can be presumed to serve.

3. Transvascular exchange; the interendothelial cleft

One of the important functions of the blood circulation is the control of the amount of water and the state of stress in the extravascular space. A process of transvascular exchange maintains the environment of the cells of the system in a state of homeostasis. Control is exercised mainly through the microcirculation, that part of the blood stream which passes through the narrowest blood vessels, the capillaries [5].

The flow of blood is on a pressure difference (head) generated by the heart. An appreciable portion of the available pressure head is required to drive blood from one end of the capillary to the other as it gives up its oxygen and passes from the arterial to the venous side of the circulation.

It is known that the walls of the capillaries are leaky, that fluid can seep across the capillary vessel wall and that this transvascular flux goes through clefts between the endothelial cells composing the innermost sheath of the capillary wall [5]. The clefts are of a very uniform width (about 150 Å) and provide a barrier, total or partial, depending on their size, to the macromolecules in solution in the blood plasma [3]. The major macromolecular constituent of plasma is serum albumin. The albumin molecule it turns out is just small enough to pass through the cleft although in hindered fashion and the concentration of albumin in tissue is much lower than that in plasma. In addition to the hydrostatic pressure difference across the vessel wall, therefore, there is also a difference in serum albumin concentration, i.e. a difference in osmotic pressure. While the hydrostatic pressure head tends to drive water from blood into tissue, the difference in osmotic pressure, or more precisely a part of it, opposes this. The hydrostatic pressure in the capillary drops along its length, and with it the hydrostatic pressure difference, but the effective part of the opposing osmotic pressure difference stays constant. At high capillary pressures, arterial end, water is being expressed from the blood, at low pressures, venous end, it is being reabsorbed into the blood stream [5]. The regulation of tissue water content depends upon this balance. Flow through the interendothelial cleft is high and outward on the arterial end of the capillary, goes to zero and then becomes a flux into the blood stream at the venous side.

The hydrostatic pressure gradient in the cleft between adjoining endothelial cells is thus different at different points along the capillary. Yet the cleft width as observed in the electron microscope stays remarkably constant [3]. Its walls stay precisely parallel, even though the cleft is often highly convoluted and there are no structures visible that stabilize the interendothelial cell membrane distance [3].

Clearly some strong potential energy minimum must hold the channel open at a precise distance. No large formed structures are seen in the cleft but stiff connections by single macromolecular bridges would be difficult to detect. Stiffly stretched polyelectrolyte bridges ensheathed by a layer of concentrated counter-ions are, as can be shown [3], particularly effective.

A widening of the cleft would induce an increased tension in the polymer chain but would also tend to pull the counter-ions into a narrower cylindrical sheet and thus reduce the base over the cleft wall over which the pressure acts. The attraction is increased, the repulsion is reduced. The opposite happens when the cleft narrows. Both the tension in the cleft and the pressure in the ion atmosphere are large so that a very sharp minimum arises between these two tendencies. All other forces which arise here and can affect gap width in principle are negligible even if the distance between the molecular bridges is quite large, say twice the cleft width.

Such a wide separation of the bridges is necessary so as not to impede water flux excessively and so as not to become an extra factor, besides cleft width, determining the extent to which macromolecular passage through the cleft is hindered.

It is interesting that in recent microstructural work these bridges have been visualized at precisely the spacing assumed in the model [3].

One aspect that affects the polyelectrolyte bridge in this case has not yet been mentioned. A polyelectrolyte, physiologically, is functioning in an aqueous medium of physiological ionic strength, i.e. in a 0.145 M salt solution. Only a relatively high local fixed charge concentration can thus be effective in attracting additional counter-ions and expelling co-ions. The fixed charge concentration in the cylinder around the polyelectrolyte longitudonal axis, within which the fixed charges are located on the basis of the chemical structure of the chains, must thus be at least 0.145 M in order to be effective. At this concentration the fixed charges would be about 20 Å apart so that their distribution in a cylinder of diameter 30 Å, as is assumed in the model, is reasonable. Note, moreover, that at 20 Å spacing there would also be no counter-ion condensation. Furthermore this distance apart would be consistent with the bridges being O-glycosydically linked glycoprotein molecules.

4. Glycoprotein structure

Glycoproteins of the type here of interest have a protein back bone and oligomeric carbohydrate chains O-glycosydically linked to the protein as side chains. Only two amino acids are able to function in this way, serine and threonine, and in a typical glycoprotein of this type more than half of the amino acids in the chains are indeed either serine or threonine. The carbohydrate coat protects the proteins against enzymatic degradation by proteases. A variety of sugar sequences are involved and the system can imprint its special character in this way. Blood groups, for example, are so determined.

The oligomeric side chains are terminated either by sialic acid or by fucose. Sialic acid is the only charged sugar involved and the charges so added tend to be located peripherally. The first sugar in each side chain, the sugar which can make the link to serine or threonine is N-acetyl galactosamine. Per sialic acid we thus have to have at least two sugar moieties and two amino acids in the backbone chain. A two amino acid spacing is not only reasonable on the basis of the available number of serines and threonines it would also be sterically difficult to add sugar side chains at a higher density. We thus estimate that a glycoprotein contains at least 700 daltons per sialic acid.

5. The glycocalyx

This brings us to the second polyelectrolyte brush system of the endothelium. The surface of the endothelial cells which faces the blood stream is coated with end attached, actually end buried, glycoprotein chains whose sugar coated sections stick into the plasma. It has been found by Born and Palinski [6] that per 100 Å^2 of endothelial surface there are some $\nu = 50$ sialic acid molecules which can be removed from the endothelial surface by the sialic acid specific enzyme neuraminidase.

How can we conclude from this that the glycocalyx is a polyelectrolyte brush system? If it is true it would have to produce a reasonably dense segment concentration. Neighboring polyelectrolytes in the brush have to interact sterically in semi-dilute fashion in a brush. On the other hand the coating must be reasonably permeable to albumin molecules since we know that these cross steadily from blood to tissue. Let us assume, therefore, that if the volume fraction of the glycoprotein is ϕ, it would not be much smaller or larger than 0.06.

Let, the inter-glycoprotein distance on the luminal endothelial surface be $2R$, the total length of the glycoprotein chain be L and b be the average distance along the chain between carbohydrate side chains bearing a sialic acid. We can then derive the following two stoichiometric relationships to characterize the glycocalyx: For the volume fraction ϕ

$$\phi = \left[M_0/N_A(\pi R^2 b)\right]10^{24} \qquad (1)$$

where M_0 is the molecular mass associated with one sialic acid residue, 700 Da at least and for the number ν of sialic acid residues per 100 Å^2

$$\nu = \left[L/(\pi R^2 b)\right]10^2 \qquad (2)$$

where L, R and b are expressed in Å and it is assumed that the glycoprotein density is 1. N_A is the Avogadro number. From (1) and (2) we find that

$$L = (10^{22}/N_A)[\nu M_0/\phi] \quad (3)$$

which for $\nu = 50$, $M_0 = 700$ and $\phi = 0.06$ gives $L \approx 10^4$ Å or 1 μm. The same parameter values also give

$$\pi R^2 b = 2 \times 10^4 \text{Å}^3 \quad (4)$$

Hence if b is about 6 Å the inter-graft distance on the surface is about 60 Å. We were thus correct in thinking that the glycocalyx is a fairly dense brush of very long chains. It is, moreover, immediately possible to calculate the fixed charge molarity of the brush, i.e. the molarity of sialic acid. It is $(10^{27}/(\pi R^2 b)N_A) \approx 0.1$ M, i.e. of the same order as the physiological neutral salt concentration.

If M_0 were twice as large as we have assumed, which is likely, L (eq. 3) would be twice as large and so would $(\pi R^2 b)$ (eq. 1), but the fixed charge molarity would be halved. It is known that glycoprotein molecules which have a dense sugar coat surrounding them are very stiff [7,8]. Their persistence length is likely to be much larger than $2R$ and would thus enhance the tendency of the high counter-ion concentration to impose a nematic parallel cylinder geometry.

6. Conclusion

It is interesting to ask how such a glycocalyx layer would affect the hydrodynamics of blood flow, what function it might serve in controlling the interaction of the 2 μm diameter platelets with the endothelial membrane and what role it might have when 8 μm diameter blood cell deform and interact strongly with the endothelium in their passage through the 6 μm diameter capillaries. It is clear from our discussion of the cleft that the polyelectrolyte would stiffly resist compression.

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