Abrupt Structural Changes in Polyanionic Gels Evoked by Na-Ca Ion Exchange: Their Biological Implications

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Summary: Anionic polyelectrolyte gels, synthetic or natural, undergo abrupt structural changes when the monovalent counterions in the gel, e.g., Na^+, are replaced with divalent cations, e.g., Ca^{2+}. These structural changes are caused by the preferential binding of Ca^{2+} to the polyelectrolyte chains and the ability of Ca^{2+} to form crosslinks between two neighboring chains. Strong lyotropic (Hofmeister) effects of anions, such as fluoride, phosphate, aspartate and chloride, on the repetitiveness of abrupt structural changes in the gel strands are demonstrated. These findings have important biological implications.

Keywords: excitability; gel; ion exchange; Na-Ca; polyanionic

Observations on Crosslinked Polyacrylate and Polymethacrylate gels: Discontinuous Volume Transition

The objective of the present study is to elucidate the nature of abrupt structural changes in polyanionic gels produced by application of various salt solutions. We have started our study along this line with the observation of a discontinuous volume transition in small beads of crosslinked polyacrylate gel induced by Na-Ca ion-exchange [1-3].

Figure 1, left, shows an example of our observations. Here, a number of small spherical gel beads of approximately the same diameter were selected under a dissecting microscope. The selected beads were introduced into a dozen cuvettes containing a given volume of 40 mM NaCl solution (at pH 7.4). Then, small aliquot portions of a concentrated $CaCl_2$ solution were added successively to the cuvettes, and the diameters of the beads were determined repeatedly until equilibrium was reached between the gel beads and the salt solutions in the cuvettes.

It is seen that addition of Ca-salt to the surrounding medium brought about a gradual decrease in the gel diameter initially. When the Ca-Na mole ratio in the surrounding solution was raised to about 1/30, there was a discontinuous, roughly 10-fold change in the bead volume. A further rise in the Ca-Na mole ratio did not produce any significant change in the gel volume.

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Very similar results were obtained by using small pieces of crosslinked polymethacrylate gel. It was possible to obtain similar results by using Li, K, Rb, Cs and NH_4 salts in place of Na salt in these observations. Mg, Sr, Ba, Co or Ni salt could also be used instead of Ca salt. Obviously, the discontinuity in the swelling curve is a consequence of the process of monovalent-divalent cation-exchange occurring in fully neutralized polyanionic gel beads. As expected, substitution of the chloride ions in the ambient medium with bromide or ethylsulfate ions had no effect on the results. The whole process is reversible: that is, the discontinuity in the swelling curve could be observed when gel beads in their shrunken state were placed in a small quantity of a $CaCl_2$ solution in the cuvette initially and a 40 mM NaCl solution was added progressively to the solution.

The important question arises. How is this discontinuous volume transition produced by Na-Ca ion exchange taking place in the gel? As the first step toward answering this question, we have examined the progression of Na-Ca exchange within the gel bead associated with a gradual rise in the Ca-ion concentration in the surrounding medium [2, 3]. In spite of the limitation to the quantity of available samples of Na and Ca in these small gel beads, we could obtain reasonably reproducible results. An example of the results obtained is shown in Figure 1, right.

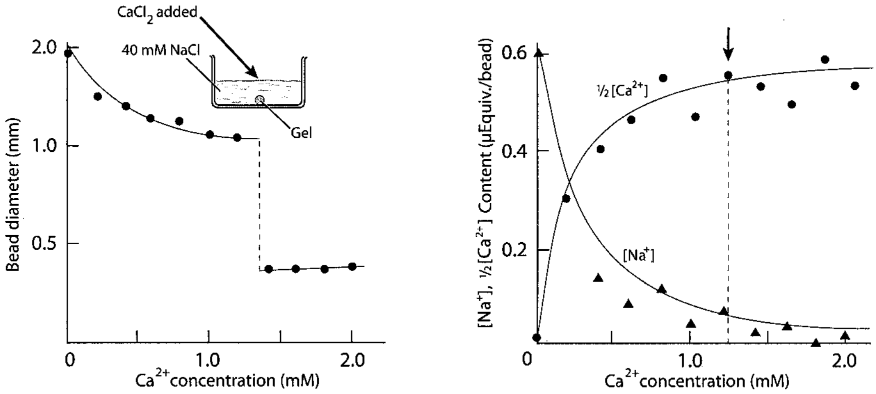

Figure 1. Left: The effect of Na-Ca ion-exchange on the diameter of crosslinked polyacrylate gel beads. As shown in the diagram on the top, the beads were immersed in a 40 mM NaCl solution, to which a small amount of $CaCl_2$ was added. Note that there was a discontinuous decrease in the gel diameter when the Ca^{2+} concentration in the surrounding solution was raised to about 1.2 mM. Right: The equivalent content of Na^+ and Ca^{2+} in the gel bead is plotted against the Ca^{2+} concentration in the surrounding salt solution. The arrow indicates the threshold Ca^{2+} concentration. The gel beads saturated with Ca^{2+} had a diameter of about 0.48 mm and contained roughly 0.3 μmole of Ca^{2+}.

It is seen that the amount of Ca in the gel rose precipitously as the external Ca-ion concentration was raised in small, gradual steps. Simultaneously, the amount of Na-ions in the gel was shown to fall in proportion. Note that, under the present experimental conditions, the density of the negative fixed changes in the gel is high enough to effectively exclude the co-ions (Cl^- in this case) from the gel. Thus, it is expected that a gain of Ca-ions by the bead is compensated by a simultaneous loss of roughly an equivalent amount of Na-ions.

It is to be noted here that the polyacrylate gel bead has great ability to take up Ca-ions against the background of the relatively high concentration of Na-ions in the surrounding solution. Note that, when the discontinuous transition is about to take place, the concentration of the Ca-ions in the surrounding solution is well below (roughly 1/30) that of Na-ions. Under these circumstances, the Ca content in the gel has risen already to roughly 80% of the final level and is far (about 4 times) higher than that of the Na-ion. It is evident that such a gigantic preference for Ca^{2+} over Na^+ can not be attributed simply to the presupposed electrostatic potential difference across the gel surface.

There is one more noteworthy feature in the figure. That is, the discontinuous transition is accompanied by a drastic (about 10-fold) rise in the average cation concentrations in the gel due to the sharp decrease in the gel volume. However, our experimental data did not indicate the presence of any detectable change in the total quantity of Ca-ions in the gel in association with this transition. Thus, it appears that the discontinuous volume transition represents the occurrence of a drastic change in the water content of the gel bead associated with a sudden change in the mode of binding of Ca ions to the anionic polymer chains.

It is well known in literature that the properties of polyions are, at higher degree of neutralization, strongly influenced by the binding of counterions.[4] Experimental data indicated that, in a fully neutralized polyacrylic acid, the osmotic coefficient of the monovalent counterions is only about 0.2.[5] Transference experiments on Na-polyacrylate showed that roughly 60% of the Na ions are associated with the polyions.[6] Obviously, the electrostatic interaction of Ca-ions with polyanionic chains is much stronger than that of monovalent cations. It is known that Ca-ions are preferentially bound to multidentate anions including carboxylate groups, and such complex-ion formation is governed by the principle which determines the formation of insoluble salts.[7] Ca-ion is a very good crosslinker between organic molecules[7] and that such crosslinking brings about profound dehydration in association with a reduction of the effective charges of Ca^{2+} and COO^-

groups.[8] On these grounds, it appears reasonable to assume that a rapid spread of the Ca-crosslinking procession along the anionic polymer chains in the gel lies at the base of the process of discontinuous volume transition.[2]

We have noted that addition of an organic solvent, such as acetone, n-butanol, etc., to the surrounding salt solution significantly lowers the threshold Ca-Na mole ratio. This finding indicates that the formation of Ca-crosslinks between anionic polymer chains is significantly promoted in the hydrophobic environment created by these organic solvent molecules in the gel beads.

A rise in the temperature also favors the appearance of shrunken state of the gel. That is, when the temperature is raised from 20°C to about 55°C, there was about a 50% decrease in the threshold Ca-Na mole ratio. This finding is indicative of the fact that the trasition from a swollen to shrunken state of a polyanionic gel is an endothermic process. [Our present estimate of the enthalpy change is roughly +2 kcal per mole of Ca^{2+}.] This endothermic process is considered to be driven by an entropy increase associated with the release of water molecules in the solvation shell around Ca ions and negatively charged polymer sites. Since the density of water in the solvation shell is higher than the density of the bulk water, the release of water molecules from the solvation shell is expected to be accompanied by a measurable increase in the volume.[9, 10]

Our exploratory dilatometric measurements have clearly indicated that there is a measurable increase in the total volume (i.e. the sum of the volume of the gel suspension and that of the added $CaCl_2$ solution) when a Na-rich, swollen gel is converted into its Ca-rich, shrunken form. A Linderstrøm-Lang and Lanz type dilatometer [11] we employed had the following dimensions. The capacity of the main chamber was 55 ml, the side chamber was 20 ml in capacity, and the diameter of the capillary was 1.4 mm. The cross-linked Na-polyacrylate gel was synthesized by the procedure described previously.[12] The gel was fully swollen in a slightly alkaline NaCl solution, then thoroughly washed with distilled water, and finally dried in the oven kept at 75°C after crushing the gel to small pieces. An aliquot portion of the dried gel grains were suspended in 35 ml of 150 mM NaCl in the main chamber. The side chamber was filled with 15 ml of 0.5 M $CaCl_2$ solution. The top portion of the chamber was filled with light mineral oil (Fisher Chemicals). After removing air bubbles in the chambers by using a vacuum pump, the dilatometer was introduced into a thermostat kept at 23 ± 0.0005°C and measurements were made of the volume changes resulting from mixing the two solutions in the dilatometer.

The results obtained are shown below:

Na-PAA	0	110	220	330 mg
ΔV	-8.6	+0.8	+8.3	+16.0 μl

where Na-PAA represents the dried sample of Na-polyacrylate gel, ΔV is the volume change, each being an average of 5 measurements (± 0.5 μl). The volume change observed in the absence of Na-PAA is very close to the value expected from mixing the 0.5 mM $CaCl_2$ solution with the 150 mM NaCl solution (see Handbook of Chemistry and Physics, 51st ed.). Our estimate of Na-acrylate monomer in the gel sample employed is close to 110 mg/mequiv. The present data, an increase of roughly 8 μl per equivalent of polyacrylate gel (crosslinked), is slightly smaller than the values extrapolated from the data published by Ikegami [8] and by Strauss and Leung [9] associated with an Na-Ca ion exchange in polyacrylate (uncrosslinked) solution.

This finding is quite consistent with the notion that the formation of Ca-crosslinks, which accompanied by a massive release of water molecules from the hydration shells, lies at the base of the process of discontinuous volume transition.

Kinetic Studies of Abrupt Structural Changes

Our recent kinetic studies of abrupt structural changes in crosslinked polyacrylate and polymethacrylate gels produced by Ca-Na ion-exchange have yielded the following results.

(1) By using a piezoelectric pressure sensor, it was possible to record repetitive changes in the hydrostatic pressure inside small gel rods in their Ca-rich state by exposing the gel surface to a Na-salt solution. These changes were characterized by an abrupt pressure fall followed by a gradual recovery.

(2) Common anions, such as fluoride, phosphate, aspartate, sulfate, chloride etc., exert strong lyotropic (Hofmeister) effects on the production of repetitive abrupt structural changes (see below).

(3) By applying a fluoride and/or phosphate salt solution of Na^+ or K^+ to the surface of a compact (Ca-rich) gel rod, electrical manifestations of abrupt structural changes occurring in the superficial layer of the compact gel layer were observed. The onset of

these structural changes was found to be extremely abrupt.

(4) The boundary between a swollen (Na-rich) and compact (Ca-rich) regions created in a fine strand of crosslinked polyacrylate gel was shown to be very sharp. By applying a weak electric current to the fine strand, it was possible to induce "propagation of swelling" along the strand.

Analogous Structural Changes in Living Nerve Fibers

It is clearly established by the method of intracellular perfusion that the excitability of the squid giant axon is strongly affected by anions introduced into the axon interior.[13] The sequence of anions arranged in accordance with the favorability for maintaining excitability was shown to be

F > HPO_4 > glutamate, aspartate > citrate > tartrate > propionate
> SO_4 > acetate > Cl > NO_3 > Br > I > SCN

It has been shown that, when anions listed near the "salting-in" (right-hand) end of the series are introduced into the axon interior, the protein molecules in the cortical layer of the axon are lost by dissolution into the internal perfusion solution, and this loss of the cortical proteins leads to a loss of excitability.[14] Dissolution of the cortical proteins does not occur when monovalent cation salt solutions of F and/or HPO_4 salts are used for internal perfusion. These "salting-out" anions are considered to enhance the attractive interaction between the dehydrated (hydrophobic) portions of the polymer chains, preventing dissolution of the proteins. It is quite remarkable to note that the axon that has just lost its ability to develop action potentials under intracellular perfusion with a 400 mM KCl solution regains excitability when the KCl solution is quickly switched to a KF or K_2HPO_4 solution (at pH 7.4).

It is equally remarkable to see that the "lyotropic" effect of common anions on the production of abrupt structural changes in synthetic gel strands is practically the same as the effect on the process of action potential production in living nerve fibers.

We have seen that the ability of a compact (Ca-rich) gel strand to develop repetitive abrupt structural changes which has been suppressed by NaCl solution can be restored by the use of a NaF and/or Na_2HPO_4 solution. While it is obvious that the macromolecular structures of nerve fibers and synthetic gel are grossly different, as Klotz [15] said regarding the effects of common anions, "there are remarkable common features in the interaction of all these macromolecules, natural or synthetic, with water".

When the effects of anions in the axon interior became clear, it was not difficult to clarify the roles played by Na^+ and Ca^{2+} in the process of nerve excitation and conduction.

(1) It is possible to evoke propagated action potentials in squid giant axons that are internally perfused with Na-salt solution and immersed in a Ca-salt solution. [2] There is no Na^+ outside the axon in this case; and there is no K^+ inside. The cortical layer of the axon is sandwiched between the Ca-salt solution outside and the Na-salt solution inside. Under these circumstances, the cortical layer of the axon can be induced to undergo transitions between the Ca-rich (resting) state and the Na-rich (active) state by application of electric current pulses, temperature changes, or various mixtures of Ca-salt solution and monovalent cation salt solution.

(2) In all the nerve fibers and cells examined, the production of action potentials is accompanied by swelling of the fibers and cells simultaneously with the rising phase of the action potential. Hence, the electric responses of nerve fibers and cells can be regarded as electric manifestations of the Na-Ca ion-exchange process.

(3) The boundary between the resting and active portion of the axon is very sharp. By solving the classical cable equation under the condition that both the potential inside the axon and its derivative are continuous at the boundary, the following equation relating the conduction velocity, *v,* to the electric parameters of a nonmyelinated nerve fibers has been derived [12]:

$$v = \frac{1}{C}\sqrt{\frac{d}{8\rho R^*}}$$

where C represents the membrane capacity, ρ the resistivity of the axon interior, d the fiber diameter, and R^* the membrane resistance at the peak of exctation. The typical experimental data for these parameters are: $C = 1.0\ \mu F \cdot cm^2$; $\rho = 36\ \Omega \cdot cm$; $R^* = 22\ \Omega \cdot cm^2$; $d = 0.04$ cm.

The conduction velocity calculated by use of this equation, 25 m/sec, agrees well with the observed value, 24 m/sec.

Conclusion

(1) The origin of the discontinuous volume transition in small crosslinked polyacrylate gel exposed to a mixture of Na- and Ca-salt solutions was examined by focusing on the preferential binding of Ca-ions to the polyanionic chains and the formation of Ca-crosslinks between the chains. The importance of the release of water molecules from the hydration shell is emphasized. It is suggested that a rapid spread of the process of Ca-crosslink formation along the anionic polymer chains in the gel lies at the base of the discontinuous volume transition.

(2) The lyotropic (Hofmeister) effect of anions, such as F^-, HPO_4^-, aspartate, Cl^- etc., on the production of abrupt structural changes in the compact (Ca-rich) gel rod was compared with the analogous effect these anions on the process of action potential prooduction.

(3) The close analogy of the mechanical and electrical manifestations of the abrupt structural changes in polyanionic gels to the process of action potential was pointed out.

(4) The macromolecular structure of biopolymers is vastly different from the structure of synthetic polycrylate or polymethacrylate gels. Yet, the effect of Ca-Na ion-exchange and of common anions on the conformations of these macromolecules in aqueous solutions are strikingly similar.

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