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# Repetitive abrupt structural changes in polyanionic gels: A comparison with analogous processes in nerve fibers

Ichiji Tasaki*

*LIMB, NICHD and LCMR, NIMH, Rm 3E-25, Bldg 13, National Institutes of Health, Bethesda, MD 20892-5772, USA*

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## Abstract

Repetitive abrupt structural changes can be produced in a small piece of cross-linked polyacrylate hydrogel by exchanging the divalent counter-ions in the superficial gel layer to monovalent cations. The temporal variations of the hydrostatic pressure, electric impedance and potential associated with these structural changes frequently show striking resemblance to those encountered during repetitive excitation of living nerve fibers. Common anions, such as fluoride, phosphate, aspartate, chloride, etc., produce a marked lyotropic (Hofmeister) effect on the repetitive structural changes both in synthetic polyanionic gels and in squid giant nerve fibers. Mechanical stress which brings about constraints in the polymer chains is found to facilitate the production of repetitive structural changes in the gel. In the stretched superficial layer of a synthetic polyanionic gel, a calcium-salt produces highly refractive bundles of polymer chains. In the Appendix, a crude model of ionized polymer chains that facilitates the consideration of cooperative structural changes in the gel is described.

*Keywords:* Polyanionic gel; Repetitive abrupt structural changes; Nerve excitation model

## 1. Introduction

It is well known that synthetic polyanionic hydrogels can be reversibly transformed from their swollen state to the compact state when the monovalent counter-ions, $Na^+$, $K^+$, etc., in the gels are exchanged with divalent cations, $Mg^{2+}$, $Ca^{2+}$, $Sr^{2+}$, $Ba^{2+}$, etc. (Katchalsky and Zwick, 1955; Kuhn, 1962; Tasaki and Byrne, 1992; Horkay et al., 2000). This structural transformation is very sharp; that is, when the mole ratio of monovalent to divalent cations in the gel or in the surrounding salt solution is varied by small steps, a discrete structural change is induced in the gel within a very narrow range of the mole ratio. The kinetic aspects of this cation-exchange process have not yet been thoroughly investigated.

During the last two decades, analogous structural changes in living nerve fibers—taking place in association with nerve excitation and conduction—have been extensively investigated (see Tasaki, 1999). The existence of cation-exchange processes involving $Ca^{2+}$ in nerve fibers had been known for some time (Tasaki et al., 1967). Much later, abrupt structural changes associated with these cation-exchange processes were discovered (Iwasa and Tasaki, 1980; Tasaki, 1999). We believe that the occurrence of these abrupt structural changes lie at the base of the process of nerve excitation and conduction.

The objective of the present paper is to pursue the analogy between the structural changes in synthetic anionic gels and those occurring in nerve fibers further. We could measure changes in the hydrostatic pressure inside a short cylindrical piece of synthetic gel by using the piezoelectric pressure-sensor that was employed in our previous nerve studies. It was noted during those measurements that common anions exert strong

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*Tel.: +1 301 530 1554; fax: +1 301 435 5035.
*E-mail address:* itasaki@erols.com.

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lyotropic effects (Hofmeister, 1888) on the production of repetitive abrupt structural changes in these synthetic gels.

We could record also the transient changes in the electric impedance and potential associated with the abrupt structural changes occurring in the superficial layer of a compact gel rod. We noticed that electric potential variations are strongly affected by the mechanical stress applied to the gel rod. In compact gel rods under appropriate mechanical stress, many records of the potential variations obtained closely resembled those of action potentials of nerve fibers. The inference drawn from these observations is that, in spite of a vast difference in the chemical composition between synthetic gels and nerve fibers, there are remarkable common features in the structural changes induced in association with the monovalent–divalent cation exchange process in these two systems.

In the Appendix, an extremely simple, crude model of a polyanionic gel for explaining the origin of the cooperative formation and breakage of the calcium-bridges is presented.

## 2. Material and method

### 2.1. Preparation of calcium-rich gel strands

Cross-linked polyacrylate gel strands were prepared by the following procedure (Tasaki, 2002). Partial neutralization of 15 g of acrylic acid (Aldrich Chem. Co.) was carried out by addition of 6 g of NaOH dissolved in 50 ml distilled water. Then, 50 mg of N,N′-methylene-bis-acrylamide (Sigma Chem. Co.) was added to the solution and, by stirring the solution with nitrogen gas bubbles, the cross-linker was completely dissolved. After addition of about 50 mg of ammonium persulfate (Aldrich Chem. Co.), the solution was drawn into about 40 mm long glass tubes of which the inside diameter ranged between 0.5 and 2.5 mm. Gelation was accomplished by keeping the glass tubes containing the pre-gel solution at 78 °C for about 1 h. To convert the synthesized gel in the glass tubes into a Ca-rich form, the tubes were kept in a 100 (or 250) mM $CaCl_2$ solution for 4–14 days. Then, by holding the short portion of the gel strand protruding from the end of the tubes with a pair of forceps (sometimes after breaking the tubes in the middle), the gel strands were removed from the tubes. The gel strands were stored in 100 or 66 mM $CaCl_2$ solution for later use.

Methacrylic acid was purchased from Fluka Chemika. Strands of cross-linked polymethacrylate gel were prepared by practically the same method as described above. Compact gel strands containing $Ba^{2+}$, $Sr^{2+}$ or $Mg^{2+}$ were prepared by using chloride salts of the corresponding divalent cations in place of $CaCl_2$ in the procedure described above.

### 2.2. Measurement of pressure changes in gel rods

Transient pressure changes associated with Ca–Na ion-exchange in a gel rod were examined by using a piezoelectric sensor (Iwasa and Tasaki, 1980; Tasaki, 1999). Piezoelectric bars of the lead–zirconate–titanate type (G-1195) were purchased some time ago from Gulton Industries, Inc., NJ. They are about 15 mm long, 1.5 mm wide and roughly 0.45 mm thick, and have an extremely high electric resistance and a capacitance of about 1.5 nF between the metal layers on the two surfaces. A short wooden stylus was attached to the bar near one end, and the other end of the bar was connected to the input of an operational amplifier, OPA 128 (Burr-Brown, Tucson, AZ). The amplifier had a 3.5 nF feed-back capacitor with a parallel resistance of 10 GΩ.

The entire assembly of the piezoelectric pressure sensor, including the power supply (±12 V batteries), was enclosed in a plastic box that was carefully shielded from electric disturbances by covering it with a copper sheet. The box was firmly fixed to a rack-and-pinion system that was a part of an old microscope. To suppress the effect of mechanical disturbances in the building, the entire device (roughly 5 kg weight) was placed on a $40 \times 40\,cm^2$ wide metal plate (about 5 kg weight) that was suspended by four 70 cm long rubber tubes.

The output voltage of the operational amplifier was amplified 10 fold and was recorded with a digital recorder, Data 6000. This simple device enabled us to record rapid pressure changes in a gel rod with a time-resolution of about of 1 ms. (Note that, the RC-product being 35 s, this device is not suited for studying slow changes in the gel structure.) The sensitivity of the device was roughly 330 mg weight per volt at the input of the recorder.

The effect of a Na- or K-salt solution applied to a short piece of gel rod in its compact state was examined by using the setup diagrammatically shown on the top of the records obtained (see later). In most cases, gel rods of 0.6–0.9 mm diameter (stored in 66 or 100 mM $CaCl_2$ solution) were used for measurements. Using a pair of small scissors, a gel strand was cut into a number of 1.5–2 mm long pieces. Much care was taken to make the cut surface as close to a right angle as possible to the long axis of the rod. One piece of the gel rod was picked up with a pair of forceps and was placed vertically on a platform fixed to the mechanical stage beneath the pressure sensor. The pressure sensor was lowered from above toward the upper end of the gel rod and the flattened lower end of the stylus was brought in direct contact with the top of the gel rod. After asserting a pressure change of 0.1–0.3 g weight in response to each of three to five steps of 25 μm downward displacement of the sensor, approximately 0.5 ml of Ca-free salt solution was introduced gently into the space surrounding the gel rod on the platform. The time-course of the pressure change was registered with a Data 6000 recorder.

### 2.3. *A.C. impedance measurement*

Transient changes in the electric impedance of a gel rod associated with the Ca–Na ion-exchange in the superficial gel layer were measured with an a.c. in the frequency range between 100 and 2000 Hz. An approximately 5 mm long portion of 30 mm long platinum wire of 0.65 mm diameter was tapered down to a point and a thin layer of insulating enamel was applied to a 15 mm long portion from the sharp tip. Then, the enamel layer on a roughly 0.7 mm long portion in the middle of the insulated zone was scraped off and platinum black was deposited on the bare surface of the wire. After rinsing this Pt wire electrode with distilled water, the tip of the electrode was forcibly pushed into an about 2 mm thick compact gel rod until the tip of the electrode reached a point about l0 mm away from the point of entry.

A weak a.c was applied between this internal electrode and another platinized platinum electrode immersed in the external medium. The current intensity was adjusted to generate a roughly 20 mV (rms) potential variation between the two electrodes. This a.c. voltage was amplified, converted into non-alternating (d.c.) voltage by half-wave rectification (using a diode 1N-914 and an RC-circuit) and was recorded with a Data 6000 recorder. The change in the recorded voltage was taken as a measure of the impedance change in the superficial layer of the gel rod. (See the diagram on the top of the records presented later.) No attempt was made to separate the observed impedance change into reactive and resistive components.

### 2.4. *Detection of potential changes in compact gel rods associated with Ca–Na ion-exchange*

Several different experimental arrangements were made to record potential variations associated with repetitive abrupt structural changes that appear in the vicinity of the boundary between the swollen and compact regions of a gel rod. In most of these arrangements, potential records were taken from a small area (less than 1 mm$^2$) of the gel surface located at or near the site of application of a solution containing both NaF and $Na_2HPO_4$. When this Ca-free solution was applied to the surface of a compact gel, abrupt structural changes are generated in a number of small patches on the gel surface. Hence, the average potential variation recorded from of a wide surface area does not yield much information about the behavior of individual patches in the area. In all these arrangements, the gel rods under study were subjected to a sizeable mechanical stress. This stress is expected to bring about an enhanced alignment of the polymer chains in the gel rod.

The experimental setup employed to obtain the records presented under Results is briefly explained here. Gel rods, about 2 mm in diameter and about 10 mm in length in their Ca-rich state, were employed. The gel rod was squeezed in between two thin plastic plates that were separating a plastic chamber into two compartments. One of the plates had a small (0.5 mm diameter) round hole at its center and the other plate had a large oval (1.5 mm × 3 mm) hole at its center. A 66 (or 100) mM $CaCl_2$ solution was introduced into the compartment on the side of the large hole. A 100 mM NaF solution (mixed with phosphate buffer) was introduced into the other compartment, and the potential difference between the two Ag–AgCl agar-electrodes immersed in the salt solutions, amplified by use of an op-amp Tl072ACP, was recorded with Data-6000 recorder.

### 2.5. *Observation of highly refractive filaments inside the gel*

When a rod or plate of cross-linked polyacrylate gel in its swollen state was transferred into a salt solution containing $Ca^{2+}$, fine filaments which were clearly visible under dark-field illumination start to grow near the surface of the gel. Photomicrographs of these filaments were taken using a Polaroid MicroCam SLR camera attached to a Bausch and Lomb dissecting microscope.

## 3. Results

### 3.1. *Repetitive abrupt structural changes in polyanionic gel*

When a piece of cross-linked polyacrylate or polymethacrylate gel in its Ca-rich (compact and transparent) state is exposed to a Ca-free, Na-salt solution, exchange of the Ca-ions in the gel with Na-ions in the solution is commenced in the superficial gel layer. Our objective here is to examine the temporal pattern of the structural changes occurring in the thin superficial gel layer of the gel in association with this cation-exchange process.

We have measured, for this purpose, transient changes in the hydrostatic pressure inside the gel by use of the experimental setup illustrated diagrammatically in Fig. 1, top (see Material and Method). The records in Fig. 1, left, were obtained from a small gel rod (roughly 0.7 mm diameter about 2 mm length) of

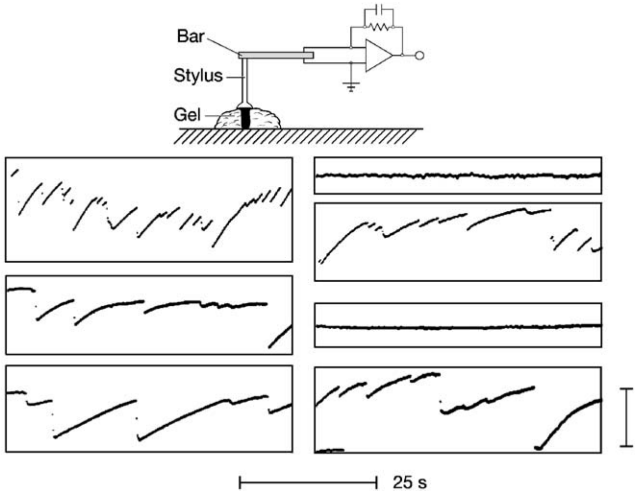

Fig. 1. Top: Schematic diagram of the setup for detecting pressure changes in a small piece of polyanionic gel rod in its compact (Ca-rich) state induced by application of Ca-free, Na-salt solution. Left: Top trace was taken from a cross-linked polyacrylate gel rod about half minute after introduction of 100 mM NaF solution. The middle trace was taken 2 min later and the bottom trace 12 min later. Vertical scale represents 40 mg weight. Right: Cross-linked polymethacrylate gel rod was used. The top and third traces were taken while gel rod was immersed in 100 mM NaCl solution. Second and fourth traces, from gel rod kept in 50 mM $Na_2HPO_4$ solution. Vertical scale, 100 mg weight, 24 °C.

compact (Ca-rich) polyacrylate gel. The rod was placed vertically on a mechanical stage located below a piezoelectric pressure sensor. By lowering the sensor gradually from above, the bottom surface of the sensor stylus was brought into firm contact with the top of the gel rod. Then, a 100 mM NaF solution (of which the pH being kept at 7.3 with Na-phosphate buffer) was carefully poured into the space surrounding the gel rod.

It is seen in the figure that, within 1 min after introduction of the Na-salt solution, the sensor started to reveal the appearance of distinct pressure variations. These variations were made up of repeating discrete downward shift of the recorder output, each representing *an abrupt fall in the pressure* followed by a roughly linear recovery. Initially, these abrupt falls were small and irregularly repeating at short intervals. During the period of following about 30 min, these abrupt pressure falls were found to become larger and more regular.

The magnitude of the abrupt fall of the force exerted by the gel on the stylus ranged from about 10 mg up to roughly 300 mg weight. The time required for recovery was far shorter than 1 s for small pressure falls, and was longer than 10 s for large falls. There was a wide variation in the time intervening between the abrupt falls; it was a few seconds in the early stage and became about 100 s or longer in the later stage. Toward the end of the 30 min period, it was occasionally seen that fairly regular series of abrupt pressure falls of nearly the same amplitude and duration were generated for several minutes. Eventually, slow variations in the pressure became the dominant feature of the sensor output.

There is little doubt that each of these abrupt pressure falls is associated with a sudden onset of structural changes resulting from the Ca–Na ion-exchange occurring in the superficial layer of the gel rod. On several occasions, we have examined the state of the gel rods under a dissecting microscope by interrupting the pressure measurement within about 15 min from the beginning. We found that most of those gel rods showed no sign of recognizable swelling. This finding indicates that these abrupt structural changes were produced by repetition of swelling and deswelling occurring in an extremely thin superficial layer of the compact gel rod.

The records in Fig. 1, right, were taken from a small piece of polymethacrylate gel rod in its Ca-rich, compact state. Here, a comparison was made of the effects of two different anion-salts, chloride- and phosphate-salt of sodium, on the production of repetitive abrupt pressure changes. First, it was shown that a 100 mM NaCl solution (with its pH adjusted to 7.3 with Hepes buffer) was totally *ineffective* in generating abrupt pressure changes repetitively. When this NaCl solution was withdrawn from the space around the gel rod and a 50 mM $Na_2HPO_4$ solution (with its pH adjusted with $NaH_2PO_4$) was introduced instead (see the second trace), the same gel rod was found to produce abrupt pressure falls repeatedly. The effect of this substitution of Cl-ions with $HPO_4$-ion was reversible (see the two lower traces).

It is of great interest to compare these experimental findings with those of our previous studies of the lyotropic effect (Hofmeister, 1888) of these anions on the interior of the squid giant nerve fiber (Tasaki et al., 1965). It was shown that phosphate is a favorable and chloride is an unfavorable internal anion for the maintenance of nerve excitability. That is, when a Cl-salt solution is introduced into the interior of giant nerve fibers, submembranous protein molecules are dissolved and released into the internal perfusion solution and this squarely leads to a loss of the excitability of the fibers (Yoshioka et al., 1978). Thus, the lyotropic effect of chloride-ions in the interior of the nerve fiber is *an enhancement of the solubility of the macromolecules*. This solubilization of the cortical macro-molecules does not occur when a fluoride or phosphate salt solution is introduced.

In the present study it was shown that, when compact gel rods are immersed in a NaCl solution, *swelling* of the gel rods become clearly visible within a period of 10–20 min. However, when these rods are immersed in a Na-phosphate solution, no swelling becomes visible during this period and the gel rods remain compact. Noting that gel swelling represents a rise in the fraction of *water molecules* in the space surrounding the polymer chains, we see that the process underlying the lyotropic effect of chloride on the synthetic gel is in essence the same as that on the gel inside the nerve fibers.

The lyotropic effects of several other anions on the abrupt pressure changes are now described. By using Na- and K-aspartate solutions (with their pH adjusted using NaOH) delivered to compact polyacrylate and polymethacrylate gel rods, it was possible to elicit repetitive abrupt pressure falls from these rods for a limited period of time. Solutions of Na- and K-citrate were able to produce a small number of repeating abrupt pressure changes in these gel rods. Only on a few occasions it was possible to record abrupt pressure changes when a sulfate-salt solution was employed. Thus, it seems safe to suggest that the sequence of anions arranged in the order of effectiveness in generating abrupt structural changes in these gels is essentially the same as that arranged according to the favorability for maintaining excitability of internally perfused squid giant nerve fibers (Tasaki et al., 1965).

Finally, it is noted that abrupt pressure changes can be evoked by application of a NaF or Na-phosphate solution in all of those gel rods prepared by using $\mathrm{Ba}^{2+}$, $\mathrm{Sr}^{2+}$ or $\mathrm{Mg}^{2+}$ instead of $\mathrm{Ca}^{2+}$.

### 3.2. *Electric manifestations of repetitive abrupt structural changes*

We now proceed to examine electric signs of the abrupt structural changes in polyanionic gel rods. The records furnished in Fig. 2 were taken from a compact ($\mathrm{Ca}^{2+}$-rich) rod of polyacrylate gel by using an a.c. (roughly 1 kHz ) for impedance measurements (see Material and Method). The a.c. was delivered across the superficial gel layer by means of a thin platinized-platinum wire electrode inserted into the interior of the gel rod. The deflection of the trace in the figure was proportional to the a.c. voltage generated between the electrode inside the rod and another Pt–Pt electrode placed in the external solution. The records were obtained between 20 and 30 min after application of a 100 mM NaF solution to the surface of the compact gel rod.

It is seen in the figure that there was a *sudden fall of impedance* followed by a very slow recovery. The amplitude of the abrupt downward deflection in the figure indicates that the impedance fell only by about 0.5–1% of its value immediately before the onset of an abrupt fall. The existence of compact gel (roughly 300 Ω cm in resistivity) between the internal Pt-electrode and the gel surface makes it difficult to assess the change taking place in the superficial gel layer. The slowness of this recovery is probably due to the absence of a sufficiently large mechanical stress in the superficial layer of the gel rod (see below).

We now examine temporal variations of the potential difference across the superficial layer of a compact polyacrylate gel rod associated with the production of repetitive abrupt structural changes. At the outset of our discussion on this subject, it should be pointed out that there is a difficult, practically unsolvable, problem in these potential measurements. Here, we are dealing with the potential difference across a cation-exchange membrane in the presence of both divalent and monovalent cations in the surrounding salt solutions. As a rule, no stirring of the salt solutions is possible. Under these circumstances, it is almost impossible to predict the *sign* and *magnitude* of the steady potential difference that is generated across the membrane (Helfferich, 1962, p. 379 and 383; Tasaki, 1982, p. 258). Furthermore, the high electrical conductivity and the great capability for swelling of a thin layer of compact polyacrylate gel present additional technical problems. Nevertheless, considering the importance of potential measurements in biology, a variety of experimental setups were employed to record temporal variations of the potential difference across polyacrylate gel layers.

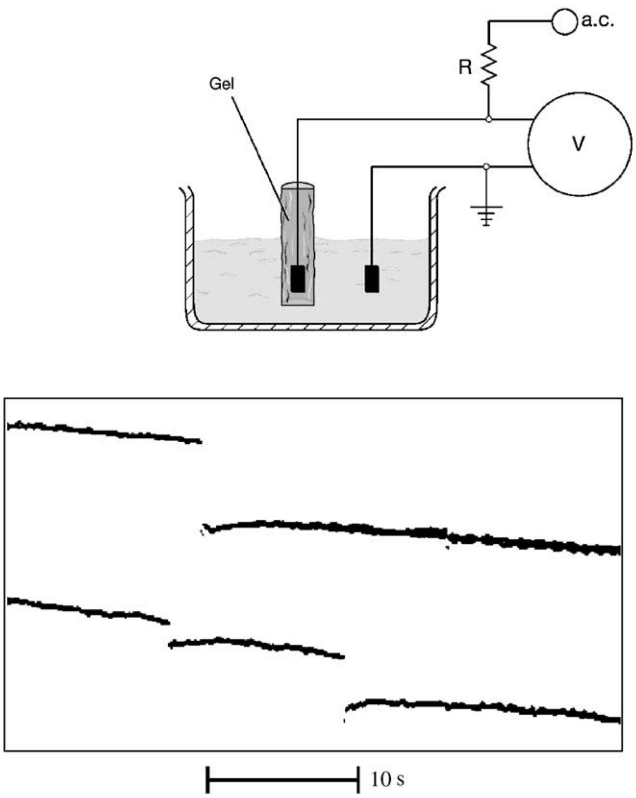

Fig. 2. Top: Diagram of the experimental setup for detecting changes in the electric impedance across the surface of a compact (Ca-rich) gel rod immersed in a 100 mM NaF solution. The impedance of the gel rod was about 1.1 k kΩ at 1 kHz. The observed change in the impedance (top trace) was about 1%. The bottom trace was taken about 30 min afterward. Note that the impedance of the gel represented by the d.c. level was continuously falling.

An example of the results of such potential measurements is presented in Fig. 3. The setup employed here is

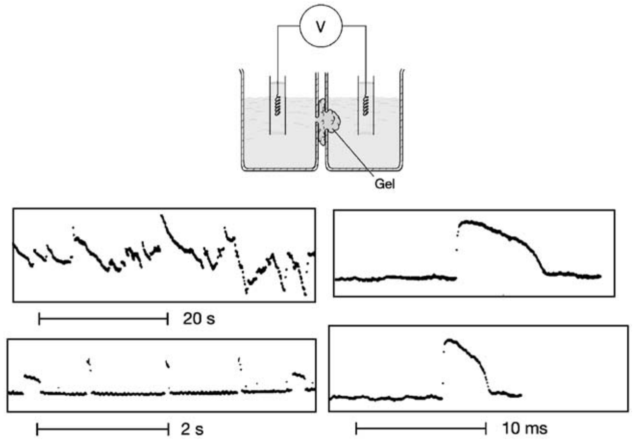

Fig. 3. Top: Schematic diagram illustrating the experimental arrangement employed for recording variations of the electric potential difference across a compact gel rod flattened by pressure. The right-hand compartment in the diagram was filled with 66 mM $CaCl_2$ solution. The records below were taken during a 30 min period following introduction of a 100 mM NaF solution into the left-hand compartment. The amplitudes of the abrupt potential rises were about 10 mV or less for the record on the left-hand top (taken immediately after the introduction of NaF), 24 and 12 mV for the trace below (taken 20 min later), and about 1 mV for the traces on the right. An upward deflection of the trace in the figure represents positivity of the right-hand electrode.

illustrated diagrammatically at the top of the figure (see Material and Method). It is seen in the diagram that a piece of Ca-rich gel was compressed by means of two thin plastic plates dividing a plastic chamber into two separate compartments. One of the compartments was filled with a $CaCl_2$ solution which was kept in contact with the gel surface by way of a relatively large hole in the plastic plate. The NaF solution, filled in the other compartment, was brought in contact with the gel surface through a small (0.5 mm diameter) hole. The variations of the potential difference between the salt solutions in the two compartments were recorded by means of a pair of non-polarizable electrodes.

The top records furnished in Fig. 3, left, were obtained shortly after exposing the gel surface to the NaF solution. Some of the records taken under these conditions appeared very similar to those taken with the pressure sensor (cf. Fig. 1, left). During the following period of 20 to 40 min, there was a gradual increase in the interval between abrupt potential jumps. From many of the gel preparations examined in this manner, short trains of almost regularly repeating potential variations which closely resembled nerve action potentials were recorded (see Fig. 3, left bottom).

It is to be noted that some of the potential variations observed under these conditions have an extremely fast rising phase (see Fig. 3, right). On a number of occasions, attempts were made to estimate the time required to complete a single (upward) potential jump. For this purpose, potential records were taken digitally on the time-base consisting of 10,000 (or 4000) points per second. The records obtained by this method indicated that the shortest time required to complete an abrupt jump was of the order of 0.1 ms under the present circumstances. Many the records showed a much slower rate of potential rise, suggesting that the underlying structural change in the superficial gel layer was spreading at a slower rate in those cases.

### 3.3. Formation of bundles of stretched polymer chains

In this section, a brief description is made of the formation of microscopically observable bundles of polymer chains induced by monovalent–divalent cation-exchange occurring in the stretched superficial layer of the gel strands. The interior of a swollen (Na-rich) gel strand immersed in a dilute NaCl (or Na-aspartate) solution is totally transparent and there is no structure recognizable under dark-field illumination (except for the faintly visible gel surface). When the solution outside the strand is replaced with a 100 (or 66) mM solution of $CaCl_2$ (or of other divalent-cation salts), the gel surface immediately becomes easily recognizable and, soon, highly refractive bundles of polymer chains start to appear in the superficial layer of the gel. These bundles originate predominantly at the freshly cut ends or some other irregular site on the gel surface. They grow in the direction along which the polymer chains are stretched in the superficial gel layer. Both the number and the thickness of these bundles increase during the following 5 to 30 min.

The left photograph in Fig. 4 shows an example of such bundles taken about 30 min after transferring a polyacrylate gel strand from a 100 mM NaCl to a 66 mM $CaCl_2$ solution. It is seen that thick, highly refractive bundles are extending from the beginning of the compact region to its swollen part of the gel strand. Due to the sudden change in the diameter of the gel strand in this transitional region, the polymer chains in the superficial layer of this region are stretched in the longitudinal direction of the strand. The bundle formation occurring in the superficial gel layer produces narrow furrows on the gel surface.

The smooth part of the swollen gel surface is nearly transparent at this stage. When this gel surface is depressed with a blunt tip of a glass rod (without penetrating the surface), it is found that fine bundles of polymer chains become visible within a few seconds. Frequently, this bundle formation produces a pattern which partly encircles the depressed area of the gel surface. This finding indicates that the mechanical stress applied to the gel surface by the glass rod enhances the alignment of the polymer chains around the depressed area, which in turn facilitates bundle formations. This effect of surface depression has been observed following

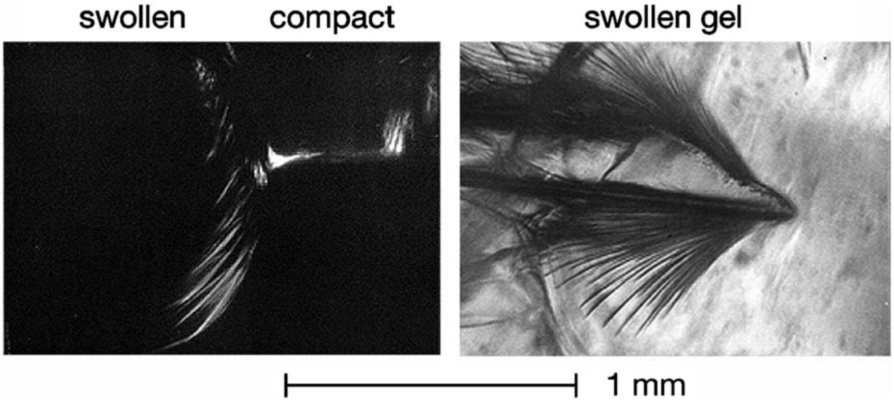

Fig. 4. Left: Photomicrograph (dark-field) of highly refractive bundles of polymer chains on the surface of a swollen polyacrylate gel rod, taken about 30 min after replacing the surrounding 100 mM NaCl solution with a 66 mM $CaCl_2$ solution. Note that the transitional zone between the swollen and shrunken (compact) regions of the gel rod is located in middle of the photograph. Right: Photomicrograph of fine bundles of polymer chains which were produced in a swollen polyacrylate gel by delivering an electric current using glass capillary containing 66 mM $CaCl_2$ solution. The current was 0.1 mA, 15 s and outwardly directed.

application of a 100 mM chloride salt solution of $Mg^{2+}$, $Ca^{2+}$, $Sr^{2+}$ and $Ba^{2+}$ to Na-polymethacrylate gel rods.

There seems little doubt that these bundles are produced by the formation of Ca-complexes bridging between neighboring polymer chains (see the Appendix). It is known that calcium is a good cross-linking agent between organic molecules, such as proteins (Levine and Williams, 1982, pp. 8, 9, 25). According to Eigen (1961), the rate of complex formation from hydrated divalent cations is an extremely rapid process.

The photograph in Fig. 4, right, shows that the process of bundle formation is greatly accelerated when Ca-ions are delivered by use of an electric current. Here, a glass pipette filled with a 66 mM $CaCl_2$ solution was pushed into a Na-polyacrylate gel rod. When a pulse of outwardly directed current was delivered to the gel by use of a Ag–AgCl wire in the pipette, there was prompt formation of highly visible bundles in the gel. The photograph in the figure was taken after passage of 0.1 mA of d.c. for about 15 s. Evidently, the pattern of the bundles represents the orientation of the polymer chains created by the insertion of the glass pipette. When an inward current was applied to the gel at this stage, the portions of the bundles near the orifice of the glass pipette were found to disappear promptly.

## 4. Discussion

We have seen under Results that abrupt structural changes can be induced in strands of compact polyanionic gel simply by external application of a $Ca^{2+}$-free, $Na^{+}$-salt solution which brings about a rapid exchange of $Ca^{2+}$ for $Na^{+}$ in the superficial gel layer. No electric current from an external source is required. Note that, in classical physiology, the standard method of inducing repetitive responses in the nerve fiber was also to replace $Ca^{2+}$ in the nerve fiber with $Na^{+}$ by external application of Na-salt of fluoride, phosphate or oxalate (Loeb and Ewald, 1916). Frequently, the temporal patterns of the potential variations associated with this Ca–Na ion-exchange in the synthetic gel strands are found to closely resemble those taken from living nerve fibers. Furthermore, the lyotropic effect of simple anions on the Ca–Na ion-exchange process in polyanionic gels is very similar to that encountered in the interior of nerve fibers.

It is obvious that there is a vast difference in the macromolecular constituents between the two systems, synthetic polyanionic gels and living nerve fibers. Yet, the conformational changes induced in these two widely different macromolecular systems by application of simple mono- and divalent cations and anions are strikingly similar. Thus, it is clear that there are remarkably common features in the interaction of these macromolecular systems, synthetic and natural, with simple cations, anions and water molecules.

Previously, we have shown that the bundles of macromolecular chains in the cortical layer of squid giant nerve fiber are aligned predominantly in the longitudinal direction and further that these bundles undergo abrupt structural transformation in association with the production of an action potential (see Tasaki, 1999). We have also known that the excitability of an internally perfused squid giant nerve fiber can be maintained only when the hydrostatic pressure inside the fiber is kept within a limited range. In the present study, we have seen that a mechanical stress applied to synthetic polyanionic gel strands enhances alignment of the polymer chains in the strand and that this alignment of the chains facilitates the production of repetitive abrupt structural changes (see Figs. 1 and 3) and the formation of bundles of polymer chains in the gel strand (Fig. 4).

It is probable that the mechanical stress constraining the polymer chains in the superficial layer of a compact gel rod exerts a strong influence on the *relaxation process* which starts immediately after the onset of an abrupt structural transition. The sudden fall in the hydrostatic pressure inside a compact gel rod induced by NaF solution (see Fig. 1) is undoubtedly the sign of an abrupt fall of the tension of the stretched polymer chains near the gel surface. During the following period, the salt composition in the environment of these polymer chains near the gel surface is expected to change with time. Note that the compact diffusion-barrier at the gel surface was disrupted at the onset of structural transition. Note also that the swollen superficial gel layer is in contact with the compact (Ca-rich) portion of the rod. The gradual recovery of the tension of these polymer chains associated with the change in the ionic environment is considered to lead eventually to a nearly complete restoration of the structure of the superficial gel layer. The temporal pattern of the responses of the gel layer to a Ca–Na cation-exchange depends on the quality and rate of this relaxation process that follows the onset of an abrupt structural transition.

## 5. Conclusion

1. Abrupt changes in the hydrostatic pressure can be induced in small pieces of compact (Ca-rich) polyanionic gel rod by application of Ca-free, Na-salt solution. The changes observed are characterized by an abrupt pressure fall followed by a gradual recovery. The repetitiveness of the abrupt pressure falls is strongly affected by the lyotropic (Hofmeister) effect of the anion species in the solution, fluoride, phosphate, aspartate, sulfate, chloride, etc.
2. The electric manifestations of the structural changes in polyanionic gel strands frequently bear close resemblance to the analogous changes observed in nerve fibers.
3. Highly refractive bundles of polyanionic polymer chains can be formed following application of a Ca-salt solution to the surface of swollen (Na-rich) polyanionic gel strands.
4. A simple, crude model of anionic polymer chains is described in an attempt to explain the origin of cooperative structural changes associated with Ca-Na ion-exchange in gel strands (see the Appendix).

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## Appendix. A theory of cooperative structural transition in an ensemble of polyelectrolyte chains

Let us examine the behavior of an ensemble of anionic polymer chains located in the superficial layer of a thin strand of cross-linked polyanionic gel immersed in a large volume of a dilute, weakly alkaline Na-salt solution. In this swollen and longitudinally stretched state of the layer, the majority of the polymer chains located near the surface of the gel strand are running roughly parallel to one another in the longitudinal direction. Since the acid groups of the chains are almost completely neutralized with NaOH under these conditions, the polymer chains are surrounded by layers of partially or wholly ordered water (dipole) molecules (see Ikegami, 1964), and a considerable portion (60–70%) of the Na-ions in the gel are loosely associated with the polyanionic chains (see Kern, 1939; Huizenga et al., 1950).

We now add calcium-salt to the surrounding sodium-salt solution in small steps. At the beginning, the Ca-ions added will be associated with individual polymer chains. As the Ca-ion concentration in the solution approaches a certain level, a Ca-bridge will be formed between some of the closely positioned pairs of the chains in the layer. Let us examine here how bundles of polymer chains are created by a gradual rise of the Ca-ion concentration under these circumstances.

It is to be noted, at the outset, that the formation of the first Ca-bridge between two neighboring chains in the gel layer is difficult owing to the restriction of the freedom of motion of the chains (decrease in the entropy). Once the first bridge is formed, however, the formation of the second bridge between the neighboring charged sites of the same pair is much easier, because these sites are located at positions more favorable for bridge formation. In other words, the first bridge, that is formed with difficulty, acts as a *nucleus* for the cooperative formation of the second, third, … bridges. This situation is completely analogous to the process of hydrogen bonding associates with structural transformation in a protein molecule (Zimm and Bragg, 1959).

Let us now proceed to analyse this process of Ca-bridge formation by the eigenvalue method of evaluating the partition function (Kramers and Wannier, 1941; Zimm and Bragg, 1959). The configuration of a pair of anionic polymer chains is described by a sequence of states, either bonded by Ca-ion or left unbonded, along the chains. We assume that these configurations can be assessed in accordance with the probability determined by the following statistical weights. The weight for an unbonded pair is arbitrarily chosen to be unity. The factor $s$ is assigned to the statistical weight for a bonded pair that follows another bonded pair. We assume that this factor $s$ is directly proportional to the absolute activity of the Ca-ions in the gel. Taking the difficulty of forming the first bridge into account, the statistical weight attached to a bonded pair that follows an unbonded pair will be distinguished from the weight of the bonded pair following another bonded pair. For the formation of every bridge that follows an unbonded pair, the quantity $\sigma s$, where $\sigma \ll 1$, is assigned. The Boltzmann factor arising from the bond energy is included in this factor $s$.

Let us consider a pair of long chains, each having $n$ negative sites. The probability of finding a configuration with $m$ bonded pairs, of which $m'$ are following unbonded pairs, is assumed to be given by

$$\frac{1}{Q}\sigma^{m'}s^{m}, \tag{1}$$

where the normalizing factor

$$Q = \sum_{\text{all configurations}} \sigma^{m'}s^{m} \tag{2}$$

is the partition function.

Let $\theta$ be the fraction of the bonded pairs in the $n$ pairs of negatively charged sites. Since $\theta$ is the expectation value of $m/n$, it follows from Eqs. (1) and (2) that

$$\theta = \frac{1}{nQ}\,s\frac{\mathrm{d}Q}{\mathrm{d}s} = \frac{1}{n}\,s\frac{\mathrm{d}}{\mathrm{d}s}\ln Q. \tag{3}$$

Now, it can be shown (see Zimm and Bragg, 1959) that the partition function $Q$ in the present case can be adequately represented by

$$Q = (1\,1)\,\mathbf{M}^{n}\begin{pmatrix}1\\0\end{pmatrix}, \tag{4}$$

where the transfer matrix $\mathbf{M}$ is defined as

$$\mathbf{M} = \begin{pmatrix}1 & 1\\ \sigma s & s\end{pmatrix}. \tag{5}$$

The eigenvalues of $\mathbf{M}$ are the solutions of the characteristic equation

$$\begin{vmatrix}1-\lambda & 1\\ \sigma s & s-\lambda\end{vmatrix} = 0 \tag{6}$$

and are given by

$$\lambda_{\pm} = \frac{1}{2}\left\{(s+1) \pm \sqrt{(s+1)^2 - 4s(1-\sigma)}\right\}. \tag{7}$$

When the matrix $\mathbf{M}$ is diagonalized, Eq. (4) becomes

$$Q = c_{+}(\lambda_{+})^{n} + c_{-}(\lambda_{-})^{n}, \tag{8}$$

where $c_{+}$ and $c_{-}$ are constants which are independent of $n$.

From a microscopic viewpoint we are dealing with very long chains with large $n$. In this case, we can omit the smaller eigenvalue in Eq. (8) and set $Q = c_{+}(\lambda_{+})^{n}$. By substituting this into Eq. (3) and using Eq. (7), the desired fraction $\theta$ is found to be given by

$$\begin{aligned}\theta &= s\frac{\mathrm{d}}{\mathrm{d}s}\ln\lambda_{+}\\ &= \frac{s}{(s+1)+\sqrt{(s-1)^2+4\sigma s}}\\ &\quad\times\left\{1+\frac{(s-1)+2\sigma}{\sqrt{(s-1)^2+4\sigma s}}\right\}.\end{aligned} \tag{9}$$

The continuous lines in Fig. 5 show the fraction of Ca-bridges formed, $\theta$, plotted against the statistical weight for a bonded pair, $s$, calculated by using Eq. (9) for two different values of the weighting factor for Ca-bridge formation, $\sigma$. Note that $s$ is proportional to the absolute activity of $\mathrm{Ca}^{2+}$ in the medium around the polymer chains. As $s$ is increased gradually from a small value, the fraction $\theta$ changes smoothly from a value close to zero up toward unity. The transition becomes very sharp as $\sigma$ is decreased.

We now turn to the macroscopic behavior of the whole gel rod. As the Ca-ion concentration in the medium surrounding the strand rises, new Ca-bridges are expected to be formed across many other pairs of polyanionic chains in the gel. Furthermore, since the polymer chains are all covalently cross-linked, the process of bridge formation is expected to spread preferentially to the chains in the immediate neighborhood of the pairs where the bridge formation is initiated (see Fig. 4). Eventually, a transition of the whole gel rod from its swollen to the compact state will be completed.

Next, let us examine the behavior of the superficial gel layer in its transitional stage. It is to be noted that, in the

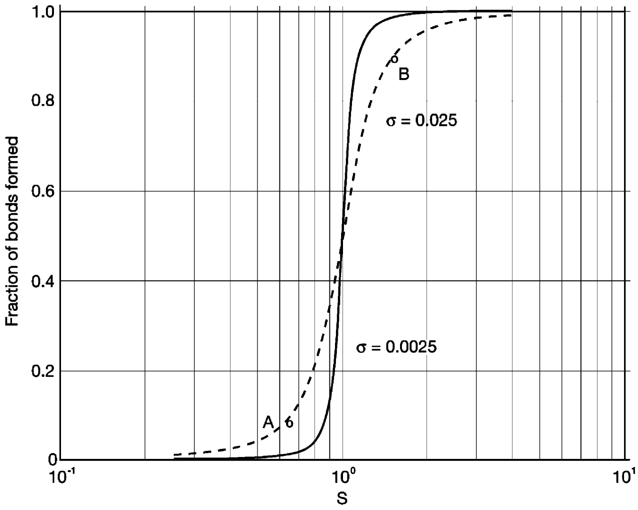

Fig. 5. Fraction of pairs of the negatively charged sites cross-linked by $\mathrm{Ca}^{2+}$, $\theta$, plotted against the statistical weight, $s$, which is proportional to the absolute activity of $\mathrm{Ca}^{2+}$ in the gel. The continuous line in the figure represents the results of calculated using Eq. (9) for the initiating factor $\sigma = 0.0025$ and the broken line for $\sigma = 0.025$.

model of polymer chains described above, transitions of charged sites from an unbonded state to a bonded state do not proceed in a random fashion. Here, there is a strong probability that the pair of sites next to an unbonded pair is occupied by another unbonded pair and, analogously, the neighboring sites of a bonded pair are taken by bonded pairs. As a consequence, sharp boundaries tend to appear between the regions of the gel occupied by unbonded pairs and the remaining regions occupied by bonded pairs (see Zimm and Bragg, 1959).

Keeping this property of the gel in mind, let us now examine what happens in the superficial layer of a $Ca^{2+}$-rich, compact gel rod when it is transferred into a $Ca^{2+}$-free, Na-salt solution. At an early stage when the fraction $\theta$ in the layer is reduced to a level slightly lower than unity (see point B in Fig. 5), the surface of the gel rod must be covered with a number of small, swollen areas (which are occupied predominantly by unbonded pairs of sites) surrounded by a wide, compact area (that is occupied by bonded pairs). Under these circumstances, a non-equilibrium phenomenon (which cannot be expected from our crude model directly) emerges in the superficial gel layer. Owing to the fall in the electric resistivity and potential difference across the swollen portion of the superficial gel layer (see Figs. 2 and 3), electric currents are generated between the swollen and compact areas of the gel surface. As in nerve fibers under analogous situations (see Tasaki, 1982, p. 286), this 'internal electric field' is expected to greatly enhance the rate of transition of the gel surface to the swollen state.

It is noteworthy that this 'internal electric field' is far weaker under the reversed circumstances, namely, when small compact areas are surrounded by a wide swollen area on the gel surface (see point A in the figure). In the small compact areas, the electric resistivity of the superficial layer remains high; hence the currents generated across these areas are weak and are ineffective in altering the state of the wide swollen area (with a low resistance). This is consistent with the experimental fact that the rising phase of the action potential, natural and artificial, is far faster than its falling phase (see Fig. 3, right bottom).

We thus see that the simple model of a polyanionic gel presented above is capable of providing us with a new useful tool for studying the process of monovalent–divalent cation-exchange in the superficial gel layer, natural and synthetic.

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