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Electrical Characterization of Gel Collected from Shark Electrosensors

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To investigate the physical mechanism of the electric sense, we present an initial electrical characterization of the glycoprotein gel that fills the electrosensitive organs of marine elasmobranchs (sharks, skates, and rays). We have collected samples of this gel, postmortem, from three shark species, and removed the majority of dissolved salts in one sample via dialysis. Here we present the results of dc conductivity measurements, low-frequency impedance spectroscopy, and electrophoresis. Electrophoresis shows a range of large protein-based molecules fitting the expectations of glycoproteins, but the gels of different species exhibit little similarity. The electrophoresis signature is unaffected by thermal cycling and measurement currents. The dc data were collected at various temperatures, and at various electric and magnetic fields, showing consistency with the properties of seawater. The impedance data collected from a dialyzed sample, however, show large values of static permittivity and a loss peak corresponding to an unusually long relaxation time, about 1 ms. The exact role of the gel is still unknown, but our results suggest its bulk properties are well matched to the sensing mechanism, as the minimum response time of an entire electric organ is on the order of 5 ms.

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I. INTRODUCTION

Certain organisms benefit from passive electrical sense organs. The electric sense offers a unique window into neurodynamics since input to a sensory system can be precisely measured and even controlled in a laboratory setting. Paddlefish electroreception, for instance, has recently proven to be a worthy model system for the study of sensory perception, neural signal processing, and the biological manifestation of stochastic resonance [1,2].

The elasmobranchs (sharks, skates, and rays) use electrosensors to enhance prey detection, to orient themselves with respect to ambient magnetic fields, and even to detect mates [3–6]; marine species show remarkable electric-field sensitivities, with thresholds dropping below 5 nV/cm in some cases [4].

The ampullae of Lorenzini are the electroreceptive organs in elasmobranchs [7]; each specimen possesses hundreds of ampullae. The ampullae are innervated, gel-filled bulbs that connect to open pores via gel-filled canals; the canals range anywhere from 3 to 20 cm in length for marine elasmobranchs, and are ≈ 0.1 cm in diameter. Lorenzini, first describing these organs in 1678, noted that the walls of the canals are ''much thicker than what is appropriate for a simple duct,'' [8] and in fact these walls are very effective electrical insulators. Sensing cells within an ampulla are thought to amplify voltage signals via an ion-channel coupling between basal and apical membranes [9]. Voltage signals between 0.1 and 10 Hz in the gel of the ampulla result in demonstrable firing rate alterations in the primary afferent nerves of the organism [7,9–11].

The electrical properties of the gel itself have gone virtually unmapped. Waltman reported a resistivity of gel collected from several skate species of 25 Ω gm at room temperature [12]. The composition and ion content of the gel in the skate *Raja clavata* and the shark. *Squalus acanthias* were also explored [13,14]. For marine species, the gel contains the following: $\approx 97\%$ H_2O by weight; a set of large sulfated glycoprotein molecules; sodium, calcium, and chloride ions at approximately the same concentrations as seawater; and slightly elevated levels potassium ions [13,14]. The extracellular glycoprotein molecules (electrophoresis has shown masses ranging from 10^4 to well over 10^5 that of hydrogen) facilitate the gel structure, and the sulfate moieties presumably render the glycoproteins effectively charged, hydrophilic, and nonfolding.

The question as to why a uniform, elastic gel fills these organs is an open one. *A priori*, the relatively stiff gel may simply maintain the geometry of the canals, meaning a more or less constant array of sensing organs. It is also possible that the gel serves to prevent infection in an otherwise vulnerable, open structure. Here, we seek to determine if evolution has fine tuned the electrical properties of the gel to aid electroreception. In addition, the data are, to the best of our knowledge, among the first reported for a naturally occurring organic polymer gel.

II. SAMPLE PREPARATION

Samples were collected from three sharks postmortem: *Triaenodon obesus* (white tip reef), *Carcharinus melanopterus* (black-tip reef), and *Carcharodon carcharias* (white). Following the method of Doyle [14], we applied pressure in the regions of the rostrum with high density of ampullae (primarily in the buccal area, located on the ''cheeks,'' and on the dorsal side of the rostrum). This yielded ≈ 5 ml of translucent gel from the reef sharks, and 15 ml from the large (5 meters in length) subadult female white shark. Gel from ≈ 150 sense organs was collected and conglomerated for each animal.

Samples were maintained at $-20\,^{\circ}\mathrm{C}$ before and after all measurements. Overall, the electrical and magnetic proper-

ties of the gel showed no change with repeated thermal, electrical, and magnetic cycling.

One 3-gram sample of white shark gel was leached of its dissolved salts via dialysis in de-ionized water. The gel was dialyzed in a dialysis cartridge (Pierce No. 66 425, 10 000 MWCO) that attenuates the levels of low molecular weight impurities while nominally allowing no component larger than 10 kDa to escape. By volume comparison, we estimate that the ion concentrations decreased to 1/1000 of their native values. Gel electrophoresis (see Sec. V) shows that the major organic molecular components of the gel are of mass greater than 15 kDa.

III. DC ELECTRICAL TRANSPORT

We collected dc conductivity data using a four-terminal enclosed cell of 3 ml volume in conjunction with a Keithley 2430 Subfemtoamp Current Source and a 2182 Nanovoltmeter.

Results for the lowest applied electric fields are shown in Fig. 1(a). All gels displayed Ohmic behavior. The sample resistivities are constant to within experimental uncertainties for electric fields from 0.1 μV/cm to 1 V/cm. We could not reach the 5 nV/cm floor (the apparent lower threshold for marine elasmobranch sensitivity), due to contact resistance. The contacts behaved as Schottky barriers, not surprisingly, as the conduction mechanisms differ between the platinum electrodes and the gel.

Furthermore, applying transverse magnetic fields between 0 and 2 mT, we find no measurable magnetoresistivity. (While these fields are somewhat low for condensed matter physics, note that the environmental fields encountered by elasmobranchs are presumably on the order of 0.05 mT).

Varying temperature had a strong effect on gel resistivity. Resistivities increased dramatically with decreasing temperature, in keeping with other electrolyte-rich systems. We plot the results in Fig. 1(b) as conductivity vs 1/T to obtain the activation energy of the transport process. Data for four samples were fit to the familiar Arhennius expression

$$\sigma = \sigma_0 \exp\left[\frac{-E_a}{RT}\right]. \tag{1}$$

The activation energy E_a for all samples closely matched 16.1 kJ/mol, the accepted value for proton transfer [15].

Missing from Fig. 1 are data for the dialyzed sample of white shark gel. The resistivity of this sample was two orders of magnitude higher than that of the unaltered gel. However, it was also Ohmic, nonmagnetoresistive, and consistent with proton transfer as a primary transport mechanism. The elevated resistivity is presumably linked to the reduced supply of charge carriers in the ion-leached material.

We summarize the transport results in Table I, including seawater measurements for comparison. Overall, we find extremely consistent dc behavior between the ampullary gels of three shark species.

Two qualitative points are worth noting and further investigation. While the properties of the gel closely matched those of seawater, the gel consistently showed lower values

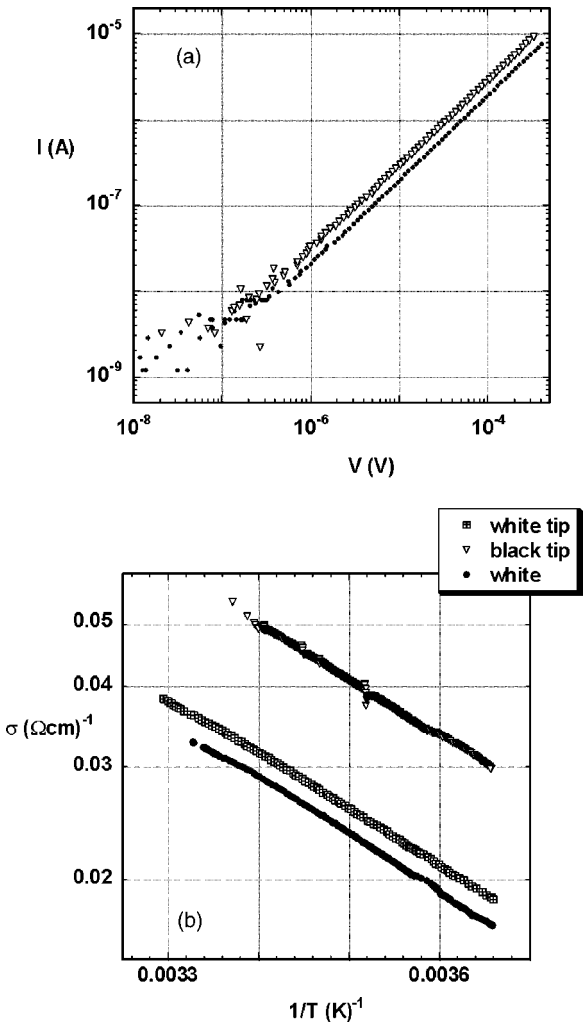

FIG. 1. Transport data collected from the ampullary gel of sharks: (a) $I-V$ traces show Ohmic response at various applied potentials, up to (not shown) 1 V; (b) semilog plot of conductivity vs reciprocal temperature for all three species. Range of temperatures matches range of possible environmental temperatures encountered by elasmobranchs (roughly 0–30 °C).

of voltage noise. In general, for repeated dc measurements, we found standard deviations of voltage signals of 40 nV for gels and 120 nV for seawater, even though the contact resistances were essentially equivalent for the two substances. While noise has been shown to enhance electroreception [1,11], the gel does not appear to be an overtly noisy electrical material.

Also, the dialyzed sample showed dramatically altered structural properties. Its volume was one-third that of the fully ionized sample, and it was qualitatively much more plastic and less cohesive than fully ionized gels. We note that structural transitions in polyampholyte gels have been observed after alterations in monomer content [16], and the volume of partially hydrolyzed acrylamide gel has shown striking electric-field dependence [17].

IV. IMPEDANCE SPECTROSCOPY

To ascertain the dielectric properties of the gel, we used low-frequency impedance spectroscopy. A Solartron 1260

TABLE I. Overview of dc measurements including bulk resistivities ($\rho = 1/\sigma$) and activation energies.

Species/sample	ρ at 293 K (Ω cm)	E_A (kJ/mol)
Triaenodon obesus white-tip reef shark	31.5 ± 0.9	16.5 ± 0.4
Carcharinus melanopterus black-tip reef shark	20.7 ± 0.6	16.2 ± 0.3
Carcharodon carcharias white shark	28.3 ± 0.6	16.2 ± 0.5
Carcharodon carcharias post-dialysis, ion-depleted sample	1220 ± 20	16.7 ± 0.5
seawater	23 ± 1	14.6 ± 0.6

Impedance/gain phase analyzer was used in concert with a Solartron 1286 electrochemical interface to collect the impedance spectra with an excitation amplitude of 25 mV. In particular, we sought the properties within the observed functional range of the elasmobranch's electric sense (0.1–20 Hz). An automatic problem with low-frequency measurements of ion-rich samples in a traditional two-plate cell is surface polarization [18]. Indeed, our measurements of the native gel were not reproducible at frequencies below 1000 Hz. To combat these effects, we collected data for the dialyzed sample. In comparing this sample to de-ionized water, we attempt to derive a basic picture of the permittivity of the glycoprotein gel.

Impedance spectra were collected as $Z = Z' + iZ''$. The sample's complex admittance follows as $Y = 1/Z$, where the real component maps the dissipative processes, and the imaginary component traces the capacitive processes. The complex permittivity is then given by

$$\epsilon' = \frac{Y''}{\omega}\left(\frac{d}{A}\right), \tag{2}$$

$$\epsilon'' = \frac{Y'}{\omega}\left(\frac{d}{A}\right), \tag{3}$$

where d is the distance between the sample electrodes, A is the area of the electrodes, and ω is the angular frequency [18]. We emphasize immediately that our admittance data do not resemble those collected from whole-organ voltage-clamp preparations, in which negative Y' values were observed [9].

Figure 2 displays data for the dialyzed white shark gel between 25 Hz and 10 kHz, where we have calibrated the effective geometry at each frequency using de-ionized water. Data were collected to 10 mHz, but surface polarization effects dominated the signal below 25 Hz and certainly influenced the measurements up to at least 100 Hz. This type of dispersion roughly fits the familiar form of a Debye dielectric [19]. The fit shown in Fig. 2 corresponds to the Debye equation

$$\epsilon(\omega) = \epsilon_\infty + \frac{(\epsilon_s - \epsilon_\infty)}{(1 + i\omega\tau_D)}, \tag{4}$$

where ϵ_s is the static permittivity, ϵ_∞ is the permittivity in the high-frequency limit, and τ_D is the Debye relaxation time. A slight emphasis has been given to higher frequencies, as these are less affected by surface polarization.

We acknowledge that the Debye scheme, with its assumption of spherical molecules, is overly simple for this sample, a gel composed of long glycoprotein molecules. Common phenomenological schemes can provide better numerical fits to the data. The Cole-Cole or Davidson-Cole approaches use power-law fitting parameters in the frequency dependence of Eq. (4), ostensibly accounting for a range of relaxation times. This would help account for the breadth of the peak in Fig. 2. However, the physical significance of these empirical exponents is debatable [20]. Mode-coupling theory has enjoyed recent success in fitting the dielectric data of polymers [21], and, given a detailed portrait of a loss peak, an array of analytical tools are available for extracting details of the polar molecules' symmetry and environment [18]. However, given the somewhat limited range of the data and the lack of detailed knowledge concerning sample structure or homoge-

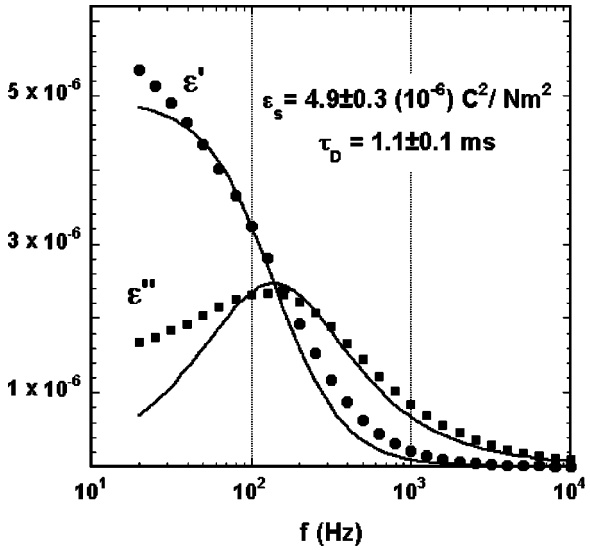

FIG. 2. Real and imaginary permittivity plotted vs frequency. The frequency-independent geometry factor was determined via an empty cell calibration assuming ϵ_0 for the permittivity of air. For our low-frequency experiments, the high-frequency permittivity, ϵ_∞, was set to that of water.

neity, we prefer to present the simplest picture at this juncture.

Two interesting features are readily apparent. The large values of ϵ' at low frequencies lead to dielectric constants ($\kappa=\epsilon'/\epsilon_0$) as large as 5.6×10^5. Such values are not uncommon for polymer samples, even in the microwave range [22], and for biological tissues [23]. While reporting absolute magnitudes for ϵ' and ϵ'' requires some confidence in the effective geometry, discerning the dominant relaxation time is more straightforward, as it is determined by the frequency of the ϵ'' peak.

The apparent relaxation time, $\tau_D \cong (2\pi f_{peak})^{-1}$, of 1.0 ms is quite long. Whether this relaxation is actually a so-called β dispersion, in which molecules rotate and align with the field, or an α dispersion, in which residual ions migrate to form an effective dipole moment within the sample, is an open question [18]. Water has its β (rotational) loss peak in the gigahertz regime, and bound water exhibits its β loss peak at 0.14 MHz, still above the range of our measurements [18]. But a long β relaxation time for the gel is not entirely surprising. Hemoglobin, a protein with a monomer mass of $\approx$64.5 kDa, has shown a β relaxation time of 0.16 μs in solution [18]. When large proteins are less than fully hydrated, they can exhibit time constants of 1 ms and longer [24].

In Debye theory, τ_D is predicted to depend on both the medium viscosity and the effective molecular volume. In addition, the β relaxation time has been shown to rise dramatically with the axis ratio of prolate ellipsoidal molecules [18]. Therefore, a long relaxation time is not out of the question considering (a) the size of some glycoprotein molecules (e.g., hyaluronic acid, a ''simple'' glycosaminoglycan has an effective diameter of 300 nm in solution [25]); (b) the presumed linear shape of the glycoproteins; (c) and the presumably high viscosity encountered by component molecules within the gel. Even if what we report here is α dispersion, an ion migration artifact, the effect could be just as important to the electric sense.

Again, the ideal comparison would be that of native ampullary gel to seawater, and with more sophisticated techniques, we hope to present such a comparison in the future. (Low-amplitude time domain measurements will be required to significantly reduce the effects of surface polarization, and using pseudorandom noise for excitation will rule out residual ion migration as a low-frequency loss mechanism). As demonstrated in Sec. III, the conductivity of native gel will be approximately equal to that of seawater, so any difference for the two substances will primarily follow the difference in their real permittivity. In this case, we would expect the native gel to have a much longer functional relaxation times than seawater, since the dialyzed gel demonstrates substantially larger ϵ' values than de-ionized water at all frequencies measured.

V. ELECTROPHORESIS

To learn more about the nature of the glycoproteins in the samples, we used polyacrylamide gel electrophoresis with Coomassie protein staining. Figure 3 displays the results for the ampullary gel of three species, along with repeated runs for the white shark after transport measurements and after dialysis. In each case, the gel exhibits large protein-based molecules ranging between 20 and 200 kDa. Though different species exhibit little similarity, we consider the striking similarity between run *B* (white shark, immediately after sample collection) and run *D* (white shark, after dc measurements, 3 months of storage, and 2 warming/cooling cycles) to demonstrate that the basic composition and structure of the gels are robust in the face of the thermal and electrical cycling encountered during experiments. Similarly, the fact that run *E* (white shark, after dialysis) shows the same essential pattern of molecules confirms that the dialysis procedure removes dissolved salts without altering the large-molecule composition of the gel.

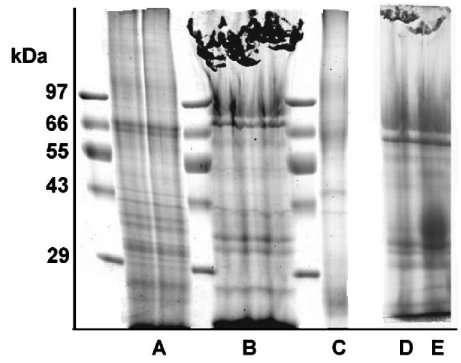

FIG. 3. Results of electrophoresis for five samples. (a) White-tip reef shark, (b) white shark, (c) black-tip reef shark, (d) white shark gel after freezing and experimentation, including electrical and thermal cycling, and (e) dialyzed white shark gel.

The origin of the high-mass anomaly (*B*, *D*, and *E*) in the white-shark trials is unknown at this time, but it is as reproducible as it is perplexing. Since the glycoproteins are known to be highly sulfated in the ampullary gel [14], the anomaly might denote massive molecules that contain a substantial effective charge.

VI. DISCUSSION: ROLE OF THE GEL

We return to the fundamental questions motivating these efforts. Specifically, we have tried to ascertain in what manner the electrical properties of the gel contribute to the electric sense. A definitive answer has not been obtained, but some suggestive aspects of the data are worth comment.

While the dc electrical properties of the ampullary gel closely match those of seawater, the impedance spectra do not. For all frequencies measured, the gel is highly polarizable, exhibiting a relatively large dielectric constant.

Similarly, the relaxation time of the gel appears to be unusually large, (on the order of 1 ms for the dialyzed gel). Why would a large relaxation time be beneficial to an elasmobranch? It would *not* assist the organ in the immediate communication of external potentials to the ampullae. Given the gel conductivities we have found, and the typical dimensions of a canal, the resistance of the pore to sensing cell path is on the order of 5–10 kΩ, hardly a good means of

communicating submicrovolt variations in the aqueous environment directly to the sensing cells of the ampullae.

However, the relaxation time reported here appears to be a good match to the response time of the entire sensory apparatus. Lu and Fishman mapped the characteristic response times of entire ampullary organs excised from skates, finding values ranging from 4 to 114 ms [9]. This is effectively the time that a voltage change in the ampullary bulb must last before it alters afferent nerve activity. In essence, a gel-filled canal may function as a low-frequency antenna that is too sluggish to respond to frequencies above 1 kHz and sluggish enough to allow the creature to neurologically register slower disturbances. An excellent conductor with negligible effective time constants (e.g., a copper-filled canal) would automatically null all pore-to-ampulla potential differences long before the elasmobranch could recognize the difference. This reasoning matches the hypothesis implemented in recent modeling efforts [26].

We repeat the caveat that impedance measurements of the native (nondialyzed) gel reliable to 0.1 Hz will be necessary to further illuminate the role of the gel.

The strong sensitivity of the gel's conductivity to temperature could help explain previous detailed reports that show remarkable similarity in thermal and electrical responses of the ampullae [27]. Thermopower of the gel could lead to significant potential differences along an electrosensory canal; in this way local temperature variations could lead directly to firing rate alterations via the electrosensory mechanism. Attempts to measure the Seebeck coefficient of the gel are underway.

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