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Biophysical Mechanisms of Cell Membrane Damage in Electrical Shock

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The phenomenon of a "magical" physical force occurring between mechanically disconnected objects has been recognized for more than 1000 years, but it was Coulomb who first defined and explained the force as "electrical." The understanding of electricity and magnetism that followed constituted the most significant scientific advance of the 19th century. Inventors such as Edison and Westinghouse realized that electrical energy could be generated in one location, transmitted by wire to another, and then used to perform work in locations at great distances from the source. The result was a complete revolution in work and lifestyles. As uses for electricity increased, it soon became clear that electrical forces can cause lethal tissue injury. The first human fatality from an industrial electrical accident apparently occurred in 1897 in Lyon, France.² Electrical injury, which has become more common since the first reported fatality, continues to present problems in clinical management. In the United States approximately 4000 individuals sustain electrical injury annually.

Peripheral nerve and skeletal muscle are more vulnerable to electrical injury than other tissues when exposed to similar field strengths. Most frequently, victims of electrical shock have at least one damaged extremity. The injury is often manifested by skeletal muscle in a rigor state surrounded by uninjured skin and may involve loss of neurologic motor and sensory functions. Approximately 85% of victims who require hospitalization have persistent neurologic symptoms that prevent their return to work. This neurologic injury pattern has been documented in many clinical reviews. A retrospective review of 64 electrical injury burn patients by Solem et al3 showed complications involving central nervous system disorders in 13% of the patients: three patients had seizures, four were obtunded, and one had quadriparesis and blindness. Peripheral neuropathies were noted in 13% of these cases. The median nerve was most commonly affected (five patients) and only partial recovery was seen in peripheral nerve symptoms. Wilkinson et al,⁴ in a review of 28 electrical burn patients admitted for care between 1973 and 1978, reported that eight patients (29%) sustained peripheral nerve injury. Four of these patients had permanent injury. In addition, the extensive

and scattered skeletal muscle necrosis and peripheral nerve damage in many electrical shock victims is a primary reason for the high amputation rate in such persons.

In a 12-year retrospective review, Varghese et al⁵ studied 85 patients admitted for burn care after an electrical accident. Thirty-four of these patients (40%) had neurologic sequelae with five cases (6%) of spinal cord damage. In patients with neurologic sequelae, the onset of symptoms was delayed. Upper motor neuron findings were documented, bony cervical injury was excluded, and partial recovery was observed in all cases. Grube and Heimbach" reported on electrical injury admissions between 1980 and 1986 in their review of 90 patients, 22 (24%) with lowvoltage electrical injury (less than 380 V) and 64 (71%) with high-voltage electrical injury. Of the 22 patients with exposure to low-voltage electric current, 11 were free of neurologic symptoms in both early and delayed periods of recovery. In the remaining low-voltage electrical burn patients, neurologic symptoms developed; these included seizures, weakness, decreased sensation, hemiparesis, and loss of consciousness. Symptoms completely resolved in 9 of these 11 patients, and symptoms improved in the other two. Of the 64 patients with documented high-voltage exposure, 21 were free of neurologic symptoms at the time of admission, although four later developed dysesthesias and shooting pains in amputated extremities. Loss of consciousness was reported in 29 of the 64 (45%) and good recovery occurred unless the loss of consciousness was associated with anoxia. About 33% of the highvoltage injury patients experienced acute peripheral neuropathies; most of these resolved. Delayed peripheral neuropathies occurred in about 20% of the high-voltage injury patients.

ELECTRIC FIELD INTERACTION WITH TISSUE

When an applied potential drop is established across the human body, mobile electrical charges will migrate and an electrical current is established. In contrast to metallic

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conductors in which the atoms are so large that outer electrons are easily swept away, physiologic salt solutions provide salt ions as the predominant charge carriers. The movement of these salt ions transfers momentum to surrounding nonionic water molecules, and the average temperature, which reflects the mean speed of the molecules, is increased. This mechanism of heating is referred to as Joule heating. Under thermally adiabatic conditions, it is characterized by a temperature rise that scales in time with the square of the current density. Under the typical industrial electrical shock conditions, the rate of Joule heating far exceeds the combined heat-dissipating mechanisms of tissue blood flow and skin surface-to-air heat transport. Thus, heat is transferred to cell membranes and they are permeabilized.

During electrical shock, the victim forms a link in a closed electric circuit. The electrical current distribution across the tissues in the body depends on the electrical conductivity of the various tissues and variation in intensity of the electrical field. In the electrical injury victim, by Faraday's law, once current travels away from the contact points to the subcutaneous tissues, it can be expected to distribute like a LaPlacian process⁷ and to distribute across the extremity tissues in such a way that the electric field strength is nearly constant in any cross-sectional plane perpendicular to the axis of the extremity.

Electric field strength is the product of the tissue conductivity and the current density (Ohm's law in continuous systems). Because of this, other factors such as variation in the area fraction of different tissues along the current path also influence the field strength.⁷ For example, when current flows through a lower extremity, the electric field strength is higher in the region of the knee and ankle than in the midcalf or midthigh, because the current density and the area fraction of bone and skin to muscle, which are highest near the joints, result in a decrease of the average conductivity over the cross-section.

Electric field strength refers to the spatial gradient of voltage (that is, volts per unit length), and it is this gradient that is important to consider in electrical injury. When a field electrician of average height is in contact with a 20,000 V power line, the magnitude of the electric field produced is likely to be highly nonuniform, but roughly on the order of 10,000 V/m. This is to be contrasted with a 110 V domestic outlet contact in the mouth of a child, where the field produced is again approximately 10,000 V/m. Knowledge of the total voltage is useful in understanding the circumstances of the injury; however, as just indicated, the local estimated electric field strength is more helpful for understanding the possibility of tissue destruction. Electrical shock simulations by computer suggest that, if perfect electrical contacts are assumed, then the root mean square electric field strength in human upper extremity tissues ranges between 60 and 160 V/cm.8

Because biologic molecules and structures are electrically polar, electric fields can exert forces that can be destructive. The field strengths just mentioned are far too small to damage biologic materials directly. However, by inducing supraphysiologic transmembrane potentials in electrically large cells, they produce much larger fields in the cell membrane. A mammalian cell placed in a physiologic solution in which a uniform electric field has been established will distort the field so that most of the ions will be driven around the cell rather than through it. The consequence is that current density in the cell is less than

that surrounding the cell. Because the density of current through the cytoplasm is much less than that surrounding the cell, the voltage drop in the cytoplasm occurs at a slower rate than in the extracellular space, and the resultant voltage drop is greater outside the cell than in the cytoplasm from one end of the cell to the other. The difference is made up by the voltage drop across the cell membrane. In general, this imposed transmembrane voltage drop scales with the size, or projection, of the cell in the direction of the field.

For a given applied field, the magnitude of the induced transmembrane potential imposed by the field depends on the cell size and orientation in the field. If the cell membrane were perfectly insulating, no current would enter the cell and the entire voltage drop would occur across the membrane. A 2 mm-long cell aligned parallel to a 100 V/cm field may experience a 10 V transmembrane potential at the cell ends. However, because 10 V far exceeds the less than 1 V threshold for membrane poration, the membrane would essentially break down before it could be fully charged to 10 V.

SIGNIFICANCE OF CELL MEMBRANE DAMAGE

Permeabilization of the cell membrane is an important mechanism of skeletal muscle and nerve injury in electrical shock. The most basic function of the cell membrane is to provide a barrier to ionic transport. To appreciate this, consider that the kinetic energy of a particle due to pure thermally driven "Brownian motion" is approximately equal to kT, where k is Boltzmann's constant and T is absolute temperature. The energy required to move solvated ions across a planar phospholipid bilayer in an aqueous environment approaches 100 kT," an indication of the membrane's strong impediment to passive ion diffusion. Because the majority of the calories used by a mammalian cell are ultimately invested in maintaining the ionic gradient across the cell membrane,10 the importance of the structural integrity of the lipid bilayer is clear. When the membrane is permeabilized, mutual diffusion of ions across the membrane increases and adenosine triphosphate (ATP)-fueled protein ionic pumps cannot keep pace. Under these circumstances, the metabolic energy of the cell is exhausted. The cell progresses to biochemical arrest and eventually to necrosis.

It is well established that the cell membrane is extremely vulnerable to physicochemical forces, such as supraphysiologic temperatures and strong electric fields.11 Even at supraphysiologic temperatures as low as 43°C, the kinetic energy of the molecules in the cell membrane can exceed the hydration energy barrier, which holds phospholipids in the membrane as a supramolecular assembly.12 In effect, the warmed membrane goes into solution in the surrounding water, rendering the membrane permeable to small ions. Electroporation of skeletal muscle cells under clinically relevant field exposure is another form of cell damage that has been shown to proceed to tissue necrosis. This nonthermal damage also renders membranes permeable to small ions. In this review, electric field interactions with tissue, nonthermal mechanisms of cell membrane damage, and thermally driven membrane permeabilization will be examined in more detail in order to indicate the specific relevant injury kinetics of each membrane damage process.



Figure 1. Mechanistic schematic of electric field-induced membrane damage. Poration of the lipid bilayer results from field-driven hydration ("electroporation") and swelling of the membrane. Strong fields also denature membrane proteins.

MEMBRANE ELECTROPERMEABILIZATION

Electropermeabilization or electroporation is commonly used to produce cell membrane defects that permit passage of molecules as large as DNA into the cell (Fig. 1). Formation of stable electropores in mammalian adult skeletal muscle cell membranes large enough in diameter to permit the flow of monovalent ionic fluorescent probes frequently results when the transmembrane electrical potential exceeds 500 to 800 mV for periods in excess of 500 µsec.13 This has become a standard technique to transfect cells that are resistant to viral and chemical methods of transfection. Electropores behave in one of two ways: they may seal spontaneously or they may be stabilized by integral membrane proteins and remain open indefinitely. If the pore spontaneously seals, electroporation is transient; if it does not, it is stable. Rupture of entire cells is probably the result of the fusion of many stable pores.¹⁴ It has been experimentally demonstrated that the probability of electroporation is determined by the magnitude of the imposed transmembrane potential and the duration for which it is imposed.15

Mammalian skeletal muscle cells may reach several centimeters in length and have electrical space constants in the range of 1 to 5 mm. Therefore, even electric fields as small as 10 V/cm may electropermeabilize these cells.¹⁶ Bhatt et al¹³ have experimentally demonstrated the occurrence of electroporation of skeletal muscle cells within intact skeletal muscle. The probability of muscle cell electropermeabilization increased with the square of the electrical field strength (Fig. 2). This membrane rupture was the direct result of the imposed transmembrane potential and was not spontaneously reversible. Striated muscle cells of human limbs are typically much larger than those of the rat. Thus, for the same applied electrical field, larger induced transmembrane potentials and a greater probability of electroporation can be expected. Also, cells tightly surrounded by other cells (that is, cells within intact muscle) are more vulnerable to damage by extracellular electric fields than isolated cells because the extracellular space provides a low-resistance current path.

There is considerably less quantitative information regarding electropermeabilization of nerve fibers. In an applied electrical field, the magnitude of the imposed transmembrane potential on a peripheral nerve axon is determined by its electrical space constant. The space constant reflects the diameter and degree of myelination of the axon. The median nerve, for example, contains 1000 to 2000 axons of several different types. When electrically shocked, the fastest axons with the largest space constant should be damaged before the slower, unmyelinated axons. Thus, the pattern of injury in a peripheral nerve following electrical injury is likely not to be homogeneous.

It has been hypothesized that electrical shock may induce the greatest transmembrane potential among nerve fibers with the largest diameter and those with noninsulating membranes.¹⁷ According to this concept, large, myelinated nerve fibers are those most susceptible to electrical injury. Large nerve fibers are the fastest conducting, and because conduction properties of nerve fibers also affect how they are damaged by electrical shocks, it is important to consider separately the damage sustained by myelinated (faster) and unmyelinated (slower) fibers. Myelinated fibers have a greater resistance than those unmyelinated.

Peripheral nerves are composed of axons that differ in size and electrical properties. The velocity of a peripheral nerve action potential depends on the diameter of the nerve fiber and the presence of a myelin sheath. The axons within a nerve trunk, which vary in size and type, therefore have action potentials of varying amplitude and velocity. The compound action potential is an aggregate of the individual axonal action potentials. Damage to subsets of the axons in a peripheral nerve can change the amplitude and shape of the compound action potential. Electrical shock may selectively injure nerve fibers of specific sizes within each peripheral nerve. Because only a subset of the

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Figure 2. Changes in muscle tissue in response to brief electric field pulses (E). The change in impedance reflects loss of membrane integrity due to electropermeabilization of skeletal muscle tissue. Rate of permeabilization is dependent upon electric field strength. Data shown reflect the electric field strength and exposure-duration (d) dependence of membrane damage.

nerve fibers may be injured, a standard nerve conduction study of nerve function may not detect the abnormality. Thus, it is important to identify the presence and degree of peripheral nerve injury reliably through other means.

ELECTROCONFORMATIONAL CHANGES IN MEMBRANE PROTEINS

Approximately 40% of the cell membrane, by weight, consists of proteins functioning as ionic channels, transporters, and signal receptors. In general, each peptide unit represents an electrical dipole moment of about 3.5 Debye (D). In an α -helical structure, which is a major functional structure of voltage-gated Na⁺, K⁺, and Ca²⁺ channel proteins, many small peptide dipoles are aligned across a cell membrane to form large dipoles with an order of 120 D. These electrical dipoles are strongly affected by external electric fields. In addition, some charged elements are movable and express as gating current during physiologic changes in the membrane potential. The logical question is, will exposure to supraphysiologic electric fields affect the function and structure of membrane proteins, particularly the voltage-gated Na⁺ and K⁺ channels, in addition to causing electroporation of the lipid bilayer?

Recent results from our laboratory suggest that membrane proteins are, indeed, damaged by imposed transmembrane potentials of the magnitude experienced by nerve and muscle in clinical electrical shock. We have observed that both Na⁺ and K⁺ channels may be damaged. Delayed rectifier K⁺ channels are the most vulnerable of the channels we have studied to date. The field-induced reduction of channel conductance and the ionic selectivity of the delayed rectifier K⁺ channels have been reported



Figure 3. Simultaneous imposed voltage (above) and measured current (below) record from skeletal muscle cell membrane. Electrical current records reflect a decrease in potassium channel conductance following electrical shock.¹⁸

(Fig. 3).¹⁸ Direct electrical shock-induced dysfunction in the voltage-gated ionic channel conductance and channel selectivity suggest possible electroconformational changes in cell membrane proteins.¹⁹

The next question is, which subgroups of the channel proteins are most sensitive to a high-voltage electrical shock? Reduction of channel conductance and ionic selectivity are primarily related to the narrowest pore of the channels, the selectivity filter. In other words, the narrowest pore of the ion channel, with a diameter of a few angstroms, is the subgroup most sensitive to an external electric field. The real mechanism for the reduction of channel conductance and ionic selectivity is unknown, but one possibility may be the reorientation of the charged particles located at the narrowest pore, resulting in an increase of interaction energy among the permient ions and the pore structure. Another possible mechanism is that supraphysiologic channel currents passing through the selectivity filter induce local heating, which results in microdamage of the molecular subgroup near the selectivity filter of the channel proteins.

In summary, electrical shock may produce voltagegated ion channel dysfunction, which may be mediated by several molecular biophysical processes and may underlie some of the neurologic findings in shock victims.

THERMALLY DRIVEN MEMBRANE PERMEABILIZATION

Thermal burn results from tissue exposure to supraphysiologic temperatures sufficient to denature macromolecules, disrupt cell membranes, and produce other effects that are typically fatal to cells. The probability of heat injury to cells is governed by both the temperature and the duration of heat exposure.20-22 The rate of heat damage to tissues is temperature dependent, a fact that was established more than 40 years ago,^{20,23} however, the relative sensitivity of different cell structures to heat damage is not yet fully documented. Thermally-driven membrane permeabilization and lysis take place at very modest supraphysiologic temperatures, 12.24.25 with a threshold value of approximately 43°C at a mean exposure time of 4 hours. At higher exposure temperatures, the mean exposure time for lysis decreases and the resultant rate of increase in membrane permeabilization decreases.

It is generally appreciated that at temperatures above 45°C inactivation or denaturation of cellular proteins occurs and that this results in cellular injury. However, the effects of temperature on the integrity of the plasma membrane have been less well studied. The kinetics of erythrocyte lysis in vitro was investigated by Gershfeld and Murayama,¹¹ who exposed red blood cells to normal and elevated temperatures for periods up to 30 hours (in vitro). For temperatures above 45°C, no hemolysis was observed. For temperatures above 45°C, hemolysis rates were rapid and the cellular damage was grossly obvious. The authors calculated an activation energy of 335 kJ/mol, a value consistent with protein denaturation or enzyme inactivation. For temperatures between 38°C and 45°C, the activation energy was much less than 121 kJ/mol.

Two different molecular mechanisms have been postulated to explain the different activation energies, which suggest that hemolysis occurring below 45°C takes place because the membrane lipid bilayer becomes unstable and begins to dissolve in the aqueous solution. Because membrane lysis occurs at temperatures below those usually required for protein unfolding, it was first postulated that thermally-induced phase changes in the cell membrane rendered the membrane less mechanically stable. In an independent laboratory, Karle²⁶ showed that there was an increase in erythrocyte hemolysis and osmotic fragility even at temperatures between 37°C and 45°C.

A second possibility, noted by Gershfeld and Murayama,¹¹ is that the structural integrity of the plasma membrane is more sensitive to supraphysiologic temperatures than are proteins. This suggestion is consistent with the idea that disruption of the lipid bilayer must overcome only the forces of hydration of the fatty acids (Fig. 4). There are no strong chemical bonds anchoring phospholipids in the bilayer. By comparison, the process of protein denaturation must alter multiple bonds, including some of the stronger bonds between segments of macromolecules. Thus, it is not suprising that the plasma membrane is one of the most temperature-vulnerable structures of the cell.

In the effort to understand the process of thermal injury at the cellular level, several investigators have focused on the thermo-tolerance of isolated cells in suspension. Lloyd et al,²⁷ Moussa et al,¹² and Rocchio²⁴ demonstrated that the membrane injury dynamics of HeLa cells and erythrocytes at supraphysiologic temperatures resemble a firstorder binary chemical reaction as described by the Arrhenius equation. The protocol in the first study of Moussa et al²² consisted of exposing suspensions of fibroblasts to supraphysiologic temperatures and then following the time course of viability. Cellular injury was presumed to occur when a bubble first appeared on the cell membrane.

Rocchio²⁴ exposed isolated rat skeletal muscle cells to elevated temperatures and measured the cell membrane permeability to an approximately 400 Da anionic fluorescent dye. In this study, the temperature of each cell was raised very quickly to a specific elevated temperature and was then maintained at a constant level while the cell was monitored for fluorescent activity. As cell membranes became unstable at supraphysiologic temperatures, dye leaked from the cells. The duration of exposure to damaging temperatures required to cause a 5% reduction of cell fluorescence was used to calculate the activation energy and reaction rate constant so that results could be compared with those of Moussa et al,22 Henriques,20 and others (Table I). Remarkably, these cellular studies are in close agreement with the much earlier clinical skin-scald burn study of Henriques and Moritz.21

The data collectively suggest that the reaction kinetics leading to thermal injury obey a simple, first-order binary chemical reaction as described by the Arrhenius expression:

$$\alpha = AE^{-E/RT(t)} = \frac{d\Omega}{dt}$$

where E is the activation energy in this single-barrier model, A is the reaction rate as $T \rightarrow \infty$, R is the universal gas constant, and T(t) is the temperature-time history of the exposed cells; α is the rate of thermal damage accumulation. The rate at which the reaction proceeds is dependent on the fraction of reactants that have at least a critical energy (E). The accumulation of injury can be determined by integrating the above equation over the time course of exposure to elevated temperatures; this is described by the parameter, Ω . The time required to produce a measurable change (corresponding to $\Omega = 1$) is entirely dependent on the parameter of observation that is chosen.

The significance of the injury parameter, Ω , when applied to an ensemble of cells or to a tissue can be appreciated by examining the simplest kinetic model. If it is assumed that cells in state X exposed to damaging tempera-

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Figure 4. Conceptual view of thermally-driven membrane permeabilization. Elevated temperatures cause membrane lipids to dissolve into surrounding water and also lead to conformational changes in membrane proteins. These structural alterations result in increased molecular permeability.

Table 1. Comparison of Experiment Results Describing Kinetics of Heat-mediated Cell Damage

Beforence	Cell Type	Temperature Range (°C)	Damage Criterion	A (1/sec)	E (kJ/mol)
Moussa et al ²² (1977) Henriques ²⁰ (1947) Cravalho et al ²⁶ (1992) Moussa et al ¹² (1979)	Fibroblasts (HeLa S-3) Epidermis Skeletal muscle Erythrocytes	45–68 45–70 45–60 44–60	Bleb_formation Epidermal cell lysis Dye leakage from cell Hemolysis (k ₁) Hemolysis (k ₂) "Ghosting" Hemolysis	$\begin{array}{l} 9.9 \times 10^{36} \\ 3.1 \times 10^{98} \\ 2.9 \times 10^{37} \\ 6.8 \times 10^{36} \end{array}$	249 628 244 250 128
Lloyd et al ²⁷ (1973) Gershfeld and Murayama ¹¹ (1968)	Erythrocytes Erythrocytes	60–76.6 38–45 45–50		2.8×10^{17} 1.8×10^{30} 1.6×10^{15}	211 121 335

tures are irreversibly altered to state Y at a rate of α , then this can be written as

$X(T) \xrightarrow{\alpha} Y(T).$

If we set the initial condition X(0) to be where X(0) indicates that all tissue is healthy, then X(t) is the fraction of tissue heat-damaged at time t. For constant temperature, the following is the solution for $\Omega(t)$:

$$\ln \frac{X(0)}{X(T)} = \Omega(T)$$

This is the simplest kinetic model for constant supraphysiologic exposure describing the ratio of surviving cells in a heated population as a function of time. When $\Omega = 1$ for a tissue, approximately 37% of cells satisfy the damage criteria. To apply this to electrical injury, the tissue temperature history is used along with the Arrhenius constants to gain an estimate of the extent of heat damage suffered by a volume of shocked tissue.

In practice, this model is used by assigning a measurable significance to $\Omega = 1$. For example, Henriques²⁰ defined it as the appearance of second-degree burn, Moussa et al²² defined it as the appearance of the first bleb on the cell membrane of fibroblasts, and Cravalho et al²⁸ defined $\Omega = 1$ as the time at which 5% of a dye has leaked from the cell membrane of rat skeletal muscle cell.

CONCLUSIONS

Damage to cells caused by electrical shock has many mechanisms. The relative contributions of these different mechanisms depend in part on the duration of current flow. If the contact time is brief, nonthermal electrical breakdown mechanisms of cell damage are most important. When contact time is longer, thermal damage predominates (Fig. 5). However, we now know that significant tissue injury can occur, under many circumstances, even when the duration of a shock is not long enough to allow for thermal damage. Additionally, the localization and characteristics of cell damage are not always consistent with simple thermal effects. A nonthermal mechanism of tissue damage, electroporation of cells in tissue, can result from electrical shock. Electroporation involves the formation of electrically induced structures in the membrane that are significantly more permeable to ions and other molecules than the normal membrane. Severe electroporation leads rapidly to cell lysis as the contents of the cell spill into the extracellular matrix. Even less severely porated cells must work harder and use more ATP to maintain membrane ion gradients essential for life. If left unchecked, this process will inexorably lead to cell death by energy starvation. Recent studies have shown that therapeutic intervention is possible by introducing molecules that seal the cell membrane, allowing time for normal cell repair processes.

Electroconformational damage to membrane proteins

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Figure 5. Nerve cells are particularly vulnerable to electric shock because of their large size, membrane electrical properties and synaptic interconnections. The primary mechanisms of damage due to electric field exposure are electroporation, thermal damage, and electroconformational changes.

was discussed as a second nonthermal biophysical mechanism of membrane damage in electrical shock. Many membrane proteins undergo conformational changes that involve movement of charge. When an intense electrical field is imposed across a membrane, the protein can be "stretched" beyond the normal configuration. This stretching may result in electrical denaturation and is somewhat different from thermal denaturation, which usually involves the loss of structural integrity of protein. We have seen in vitro that this process of protein electroconformational change can be reversed under the right conditions.

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