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Article

Action Potential and Water Molecules

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Abstract: The occurrence of potential spikes in a cell is a sign of life, and it is called action potential. There is a common notion that neuron signal conduction is the conduction of action potential. Hence, action potential is a typical and essential life activity. However, such potential spikes occur even in simple nonliving systems. According to the experimental observations by Pollack, structured water molecules can generate a negative potential environment. From this observation, the potential spike generation process for both living and nonliving systems caused by ion and water molecule adsorption-desorption process could be explained in this paper. So, taking into consideration the electrically neutral water molecules,the action potential generation mechanism could be explained. It is a fully inanimate model. Hence, the action potential may not be a life activity. Here, the role of water molecules in life is investigated further. It was found that the phase transition of the membrane is involved in the neuron signal conduction, but the membrane phase transition could be due to the change of state of the water molecules, which forms a large-scale structure in the cavities created by a number of lipids.

Keywords: membrane potential; water; adsorption; structured water

1. Introduction

The charges of ions in a cell and its environment are responsible for the characteristics of membrane potential, and this fact cannot be doubted both thermodynamically and electromagnetically. Hence, the motion of ions and its physical chemistry characteristics are seriously considered when it comes to the investigation of membrane potential.

Water is a fundamental substance for life. Hence, water is taken into consideration as an essential substance for ruling the membrane potential characteristics as well as for ions. Water plays a role primarily as a solvent for ions, and the ion concentration changes in accordance with the quantity of water. But water may have another fundamental role in membrane potential generation. G. H. Pollack is an authority of water study and has been involved in the investigation of the role of water in life [1–5]. This view is absolutely different from the ordinary life science view. There, water is studied from the view of physical chemistry and thermodynamics. Especially, the focus is on structured water. Structured water concept is not new. However, the meaning of such a water structure for life has rarely been reported. For decades, free water is an essential substance as a high fluidity solvent. More than a half-century ago, G. Ling suggested that living systems contained a great amount of structured water instead of free water, and it sustained the whole life [6,7]. Pollack agrees with Ling's suggestion and has studied structured water. Pollack has found various unusual characteristics of water which could be attributed to the formation of a visibly large water structure [2,3]. One of the most striking findings from the Pollack's study of water is the discovery of the nonzero potential generation by the formation of structured water [2,8]. If it is a genuine phenomenon, then it is a fundamental finding for understanding membrane potential. That is, water is not only a solvent for ions

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but also plays a role in generating the nonzero potential. Therefore, when discussing the membrane potential, water molecules and ions should be taken into consideration. In this work, the role of water in life is further explored.

2. Nonzero potential in the absence of ions

A 16 wt% PVA aqueous solution was prepared in a small beaker. A KCl solution (0M, 10^{-4}M, 10^{-2}M, 1M) was poured on the PVA layer gently so that the KCl solution would not mix with the PVA solution. A pair of Ag/AgCl electrodes (a reference electrode and an indicating electrode) were prepared, and both were placed in the KCl solution so that their tips were located high enough above the PVA layer. The potential generated between the pair of electrodes was measured when moving the indicating electrode downwards. Once the indicating electrode tip reached close enough to the PVA layer, the potential plunged, and further movement of the indicating electrode tip into the PVA layer detected ~ -15 mV. This potential behavior was also observed when using 0M KCl solution. So, the electrically neutral PVA solution exhibited negative potential in deionized water in the absence of ions (K^+ and Cl^-). What causes this nonzero potential? Could it be due to the formation of structured water by the water molecule adsorption on the PVA networks? Even the neutral polymer appears to be responsible for "the nonzero potential" generation. So, the formation of structured water could result in the nonzero potential, and it can be further interpreted that the collapse of structured water could lead to "the zero potential." To sum up, Eq. 1a takes place from the view of water molecule structure, and it can be interpreted as Eq. 1b in view of the potential generation.

$$\text{free water molecules} \rightleftharpoons \text{structured water molecules} \tag{1a}$$

$$\text{zero potential} \rightleftharpoons \text{nonzero potential} \tag{1b}$$

The dynamics of the action potential may be explained by taking into consideration both the ion and the water molecule characteristics, instead of just the ion characteristics only. The following sections explains further.

3. Action potential profile

Action potential generation is regarded as one of the life activities. Fig. 1 shows the action potential of a living cell and the potential spike of a proteinoid microsphere. Both are the potential traces from ref. [9,10]. Proteinoid microsphere is not a life at all, but it exhibits potential spike spontaneously, and its profile is indistinguishable from the action potential of a living cell. Figure 1 inevitably raises a question: Is action potential a consequence of life activity?

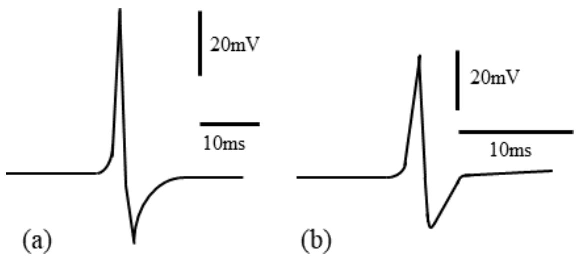

Figure 1. Potential spikes of (a) a living cell and (b) a microsphere of proteinoid Both profiles are reproduced from the ref. [9].

A simple inanimate experimental system that spontaneously generates the potential spikes, where its trace highly resembles the action potential of the living cell was executed, and is as shown in Fig. 1(a). Yoshikawa et.al. made measurements across an artificially created membrane separating 0.5M NaCl and 0.5M KCl solutions [11]. The membrane is a filter paper containing monoolein (glycerol α-monooleate). In their work, spontaneous potential spike generation was observed. Based on that work, a simple experiment was proposed and performed for this paper. Here, measurements of potential between a 0.5M NaCl solution and a 0.5M KCl solution separated by a PTFE filter (PTFE FILTER PF100 (ADVANTEC, Tokyo)) as a function time were made. The same measurement of potential was performed by replacing the PTFE filter with a glass fiber filter (GLASS FIBER FILTER GC-50) (ADVANTEC, Tokyo)). Figure 2 shows a part of the potential profiles that was obtained experimentally. Both exhibit the action potential-like profiles though the time scale is different from the living cell action potential shown in Fig. 1(a). Nonliving systems can generate the action potential-like potentials. Therefore, generation of the action potential may not be the consequence of life activities.

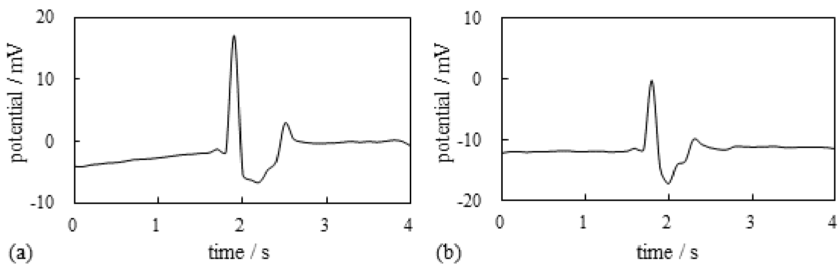

Figure 2. Potential spikes between a 0.5M NaCl solution and a 0.5M KCl solution separated by (a) a PTFE filter and by (b) a glass fiber filter

4. Contribution of water to the potential generation

As suggested in section 2, water structure could play a role in generating the nonzero potential. But how does the potential behave if formation of the water structure results in a negative potential generation? First, the relationship between the nonzero potential generation and the behaviors of the ions and water molecules is elaborated. Figure 3(a) represents the model of a water molecule. Two protons of water bear a slightly positive charge, while one oxygen atom of water bears a slightly negative charge. Therefore, the water molecule is approximated by an oval shaped substance, half of which bears the positive charge, and the rest of it bears the negative charge [1,6,7]. Figure 3(b) shows the free cations distribution in free water. If a plate bearing the cation adsorption sites is placed into the electrolytic solution, the cations are spatially fixed on the plate as illustrated in Fig. 3(c). Such localized positive charges generate a heterogeneously distributed positive potential environment. Now, what will happen when the hydrophilic plate bearing the cation adsorption sites is placed into the electrolytic solution? The cations are adsorbed on the plate, resulting in a positive potential environment. At the same time, the water molecules form a structured water layer as illustrated in Fig. 3(d). As described in the section 2, the structured water could create a negative potential environment [2,8]. Therefore, the positive potential environment formed by the spatially fixed cations must be largely neutralized by the structured water formation. Hence, both the local ion adsorption and the structured water formation could result in the nonzero potential generation, and the combination of them results in the action potential environment. With this concept in mind the potential spike generation mechanism explanation follows.

Figure 4 shows this paper's model for explaining the potential spike generation process of a living cell. Figure 1(a1) shows the resting state and the ions are localized by their adsorption on the adsorption sites, and water molecules are in the structured state. Once some external disturbance is exerted on the living cell, the structured water begins to collapse as illustrated in Fig. 4(b). Since the structured water formation causes a negative potential environment, the collapse of structured water results in a potential increase. The full collapse of structured water can lead to the highest potential generation as illustrated in Fig. 4(c). Full collapse of the structured water results in the increase of free water quantity. Hence, the adsorbed ions are liberated en masse according to the mass action law

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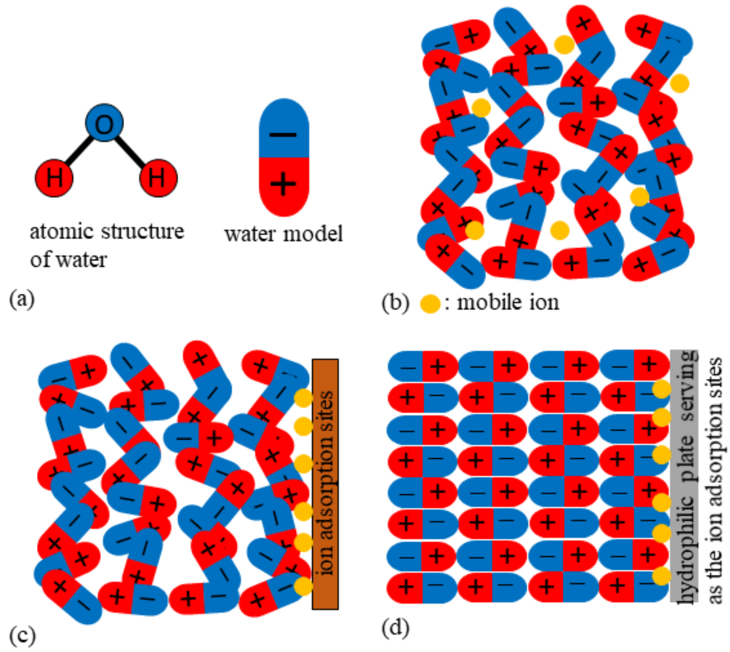

Figure 3. (a) A water molecule and its model (b) the free cations distribution in the free water (c) Spatially fixed cations by their association with their adsorption sites on the plate submerged in the electrolytic solution (d) The spatially fixed cations in the structured water phase

[6,12]. The liberated ions tend to diffuse homogeneously from the localized adsorbed state, resulting in the potential plunge as illustrated in Fig. 4(d). Then, full ion desorption and homogeneous distribution results in the full plunge of potential as illustrated in Fig. 4(e). Then again the initial state is restored as illustrated in Fig. 4(a2) (= (a1)). This potential spike generation process does not require something special such as ion channels and pumps. Therefore, it is applicable to both living and nonliving systems.

5. Role of water

"Water is the source of life" is the commonly accepted biological notion. The fundamental importance of water must lie largely in its high fluidity. Water fluidity allows transporting the essential substances for life everywhere in the body of life. If the water is not highly flowable, the water we drink won't circulate in our bodies. However, the water appears to be able to exist in the less flowable state, and it appears to play an essential role in life as exemplified by the discussion so far made about the potential spike generation. Without the structured water (less flowable water), the potential spike of life may not be generated as explained in the previous section, and such a condition must cause serious problems to life, for instance, the inability of the neuron to conduct the signal.

Such a less flowable state of water is the liquid crystal state, which is the less-known state of water other than the commonly known gas, liquid and solid states [1,4,5]. Liquid crystal state is not the solid state, but it is not the liquid state, either. It is between the liquid state and the solid state. But Pollack says that the liquid crystal water sustains life. The embryonic idea of the relationship between the liquid crystal water and life was proposed by G. Ling first more than a half-century ago [6,7]. His idea had not garnered much attention and virtually it had been ousted from the scientific community. However, Pollack had that unconventional view, and he had considered Ling's idea from his own viewpoint. He saw the liquid crystal water and life from the views not only of physiology but also of engineering. Among his new findings about water, the discovery of EZ water (Exclusion Zone water) is highly featuring. He suggests that virtually all the water in the vicinity of the hydrophilic surface is in the liquid crystal state. Pollack performed a quite simple experiment. Figure 5 illustrates his experimental system [3]. He prepared a

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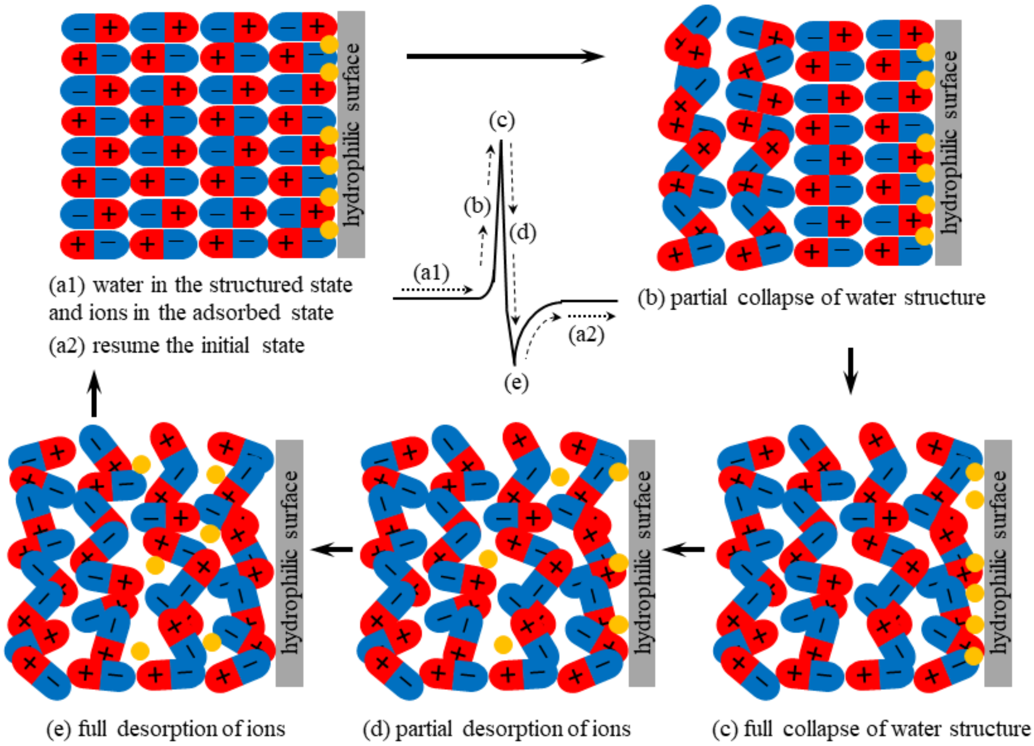

Figure 4. Potential spike profile and its corresponding status of water molecules and ions (a1) the cations are in the adsorbed state in the structure water phase (b) partial collapse of structured water phase (c) full collapse of structured water phase (d) partial desorption of cations (e) full desorption of ions ← (a2) the restoration of the initial state of (a1).

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hydrophilic polymer sheet called Nafion (DuPont, USA) surrounded by water containing a number of tiny polystyrene beads. Initially, the beads were in the homogeneously dispersed state as illustrated in Fig. 5(a). But after a while, the beads were fully excluded from the water phase in the vicinity of Nafion surface as illustrated in Fig. 5(b). The beads exclusion might be due to the formation of EZ composed of liquid crystal water.

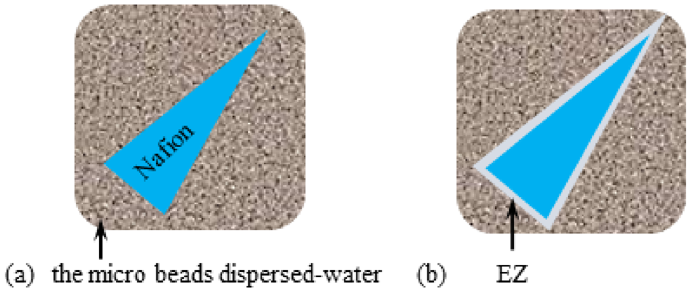

Figure 5. Formation of EZ at the boundary between water and Nafion (a) Nafion sheet placed in water containing the homogeneously dispersing microbeads (b) Formation of EZ containing the liquid crystal water only (no micro beads)

He observed the same phenomena even using hydrophilic materials other than a Nafion. Pollack proposed that the exclusion of beads was due to the formation of large-scale liquid crystal state water. So, he calls it "EZ" water (EZ = Exclusion Zone). Though still it is not completely elucidated what causes the beads exclusion, the beads exclusion genuinely takes place. Pollack observed the beads exclusion even using a real muscle as a hydrophilic material. So, the high fluidity of water is fundamentally important for life, but a large quantity of water with a lower fluidity (liquid crystal water) could exist within the living system, and it may play a certain essential role in sustaining life [1–6,13]. In connection with that, Ling suggested that the water in the living cell is highly structured (same as the liquid crystal state), and the variation of the degree of its affinity to mobile ions regulates the intracellular and the extracellular concentration of those substances [6,7]. Hence, no ion channels and pumps are needed for the ion concentration regulation of living cells nor for the action potential generation.

In 2022, a Russian science journalist, Lev Verkhovsky reported the work done by R.-H. N. Mikelsaar more than 20 years ago [14,15]. Verkhovsky suggests the fundamental importance of Mikelsaar's work for life by adding his view (Verkhovsky's work is not published in English journals, but the summary of Verkhovsky's work translated into English is available on Researchgate on the internet). Here, Mikelsaars' work based on the Verkhovsky's interpretation and his view to the Mikelsaar's work is explained as follows.

During playing with the precise molecular model of lipids, Mikelsaar found that the three phospholipids formed a right hexagonal prism. Every prism is closed above by "a hat" of three polar heads of lipids - they are bound by electrostatic interactions. Such hexagonal trimeric units cover all the surfaces of the membrane - it looks like the floor of a room with a honeycomb structure. Then it is described in the Verkhovsky's report "*But inside prisms, there are cavities which must be filled with some substance. It turned out that the three molecules of cholesterol perfectly fit it; however, the quantity of this steroid in the lipid layer can vary and be not enough to fill all prisms. In this case, the prisms can contain - and this is a clue point - tubes of structured (ice-like) water (they are named shafts); thus, a so-called hydrophobic lipid membrane may contain significant amounts of water. It is important that in the hydrophobic environment of lipid tails, this water (shafts) will not freeze at zero Celsius but at a higher temperature, possibly physiological temperature.*" This Verkhovsky's statement is thermodynamically convincing.

Verkhovsky refers to Heimberg-Jackson model of neuron signal conduction mechanism [16,17]. Heimberg and Jackson discussed the anesthesia mechanism and stated that the biomembrane exhibits phase transition and also stated that many biological membranes display melting transitions slightly below body temperature as in ref. [16]. Namely, they

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pointed out the intimate correlation between the biomembrane phase transition and anesthetic effect. Several researchers suggest the occurrence of biomembrane phase transition as well [18–20]. Probably the Verkhovsky's statement to the effect that the shafts will freeze around at physiological temperature was made with the Heimburg-Jackson model in mind. Both Verkhovsky and Heimburg & Jackson must agree on that the anesthetic effect is caused by the structure distortion of plasma membrane by the penetration of anesthesia molecules. As Verkhovsky pointed out if the shaft in the plasma membrane is responsible for the biomembrane phase transition, the anesthesia molecules must distort the membrane structure, and the shafts cannot be formed and the membrane cannot exhibit the phase transition. Such a membrane deteriorates the neuro signal conduction effect. So, even some of the neuro signal diseases might be due to the inability of plasma membrane to form the right-structure shafts.

Structured water could play an extremely fundamental role than we imagine for the neuron signal conduction as well as for the potential spike generation.

6. Conclusion

Less flowable water (structured water, liquid crystal water, ice-like water ...) has been known in the scientific community for many years. Of course, physiologists have known it. But almost no one has paid much attention to its physiological role, but only the limited number of researchers such as the late Gilbert Ling have intensively worked on the relationship between the structured water and life up until today [6,7].

This paper's work shows that PVA aqueous solution exhibits negative potential, and it is a supportive evidence that the structured water exhibits negative potential advocated by Pollack [2,8]. Taking it into consideration, we proposed the potential spike generation mechanism. Potential spike generation is not a phenomenon particular to living systems, but it can be observed in the nonliving system demonstrated in Fig. 2. The potential spike generation mechanism we proposed is an inanimate model. Hence, it does not require the functionalities of ion channels and pumps. Therefore, potential spikes of both living and nonliving systems can be explained in our single model. We further pointed out the unknown but fundamental role of water in life by referring to the works done by Mikelsaar, Verkhovsky and Heimburg & Jackson. Their works are also indirect evidences to support the fundamental role of structured water for life. Series of works done by Ling, Pollack, Mikelsaar, Verkhovsky and Heimburg & Jackson come on the same line though their views must be in some point in conflict with one another, too. For instance, Heimburg and Jackson attribute the nerve signal conduction to the mechanical wave conduction rather than the electrical potential conduction. But at least, their views to the electrical signal observed in the living systems are basically on the same line, and our work suggests that the structured water must play a role in the dynamics of electrical signal generation of life.

The inanimate model as the potential spike generation mechanism is not easily acceptable from the view of conventional physiology. But there appear to be many factors which look absent from the conventional views such as "ion adsorption", "water adsorption and structuring", "mass action law", "activity change of water by its state change, ... "Nature prefers the simplicity" but it does not mean we are allowed to oversimplify the nature. There is need to scrutinize the thermodynamical facets of ions and water molecules, and especially focus on their mass behavior instead of studying the individual molecular mechanism of ion channels, pumps and etc. The long-overlooked characteristics of water molecules must play significant role in sustaining life.

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