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GHK eq. and HH eq. for a real system is mathematically associable to each other but their physiological interpretation needs a reconsideration

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ABSTRACT

Despite the long and broad acceptance of the Goldman - Hodgkin - Katz equation (GHK eq.) and the Hodgkin - Huxley equation (HH eq.) as strong tools for the quantitative analysis of the membrane potential behavior, for a long time they have been utilized as separate concepts. That is the GHK eq. and the HH eq. have not been associated with each other mathematically. In this paper, an attempt to associate these equations to each other mathematically was demonstrated and was successful by viewing the system in question as a thermodynamically real system rather than an ideal system. For achieving that, two fundamental physical chemistry concepts, the mass action law, and the Boltzmann distribution were employed. Hence, this paper's achievement is completely within the framework of common thermodynamics. Through this work, the origin of the membrane potential generation attributed to the ion adsorption-desorption process and governed by the mass action law and the Boltzmann distribution is expressed to be plausible, whereas the existing membrane potential generation mechanism states that membrane potential is generated by transmembrane ion transport. As at this moment, this work does not intend to deny the transmembrane ion transport as a membrane potential generation mechanism but urges the readers to reconsider its validity, since this work suggests that the ion adsorption-desorption mechanism is as plausible as the transmembrane ion transport mechanism as a cause of membrane potential generation.

1. Introduction

Membrane potential generation is a consequence of ion transport across the plasma membrane in the existing electrophysiological concept, membrane theory (Ling, 1992; Ling, 2001). It is widely acknowledged that the transmembrane ion transport owes to the biological activity of the functional proteins, the ion channel, and the sodium-potassium pump which are embedded in the plasma membrane. The functioning of the pump is an indication that the cell is living since the pump consumes ATP energy (Ling, 1992; Ling, 2001). So, the generation of membrane potential is an indication of the existence of life. Further, the vehement membrane potential change, which is called the action potential, is believed to be the consequence of the transmembrane ion flow rate change caused by the functionality change of the ion channel and the sodium-potassium pump. If so, the action potential generation should be a phenomenon unique to a cell bearing the plasma membrane in which the ion channel and sodium-potassium pumps are embedded.

Contrary to such a notion, it is well-known that a potential behavior indistinguishable from the membrane potential of the living cell has been repeatedly observed in nonliving systems. One of the most typical examples is S. Fox's observation (Ishima et al., 1981; Przybylski et al., 1982; Przybylski and Fox, 1984; Haefner, 1992). Fox synthesized a protein-like polymer chain by thermally treating amino acids, called the proteinoids. Fox synthetically obtained a microsphere primarily consisting of a proteinoid and

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lecithin and found that its structure was similar to a living cell (Ishima et al., 1981; Przybylski et al., 1982; Przybylski and Fox, 1984). Microscope observations showed that the microsphere was covered with a plasma membrane-like shell and the microsphere diameter was in the micron level which was close to the dimension of a real cell (Ishima et al., 1981; Przybylski et al., 1982; Przybylski and Fox, 1984). However, the microsphere is not a living matter at all, and the shell covering this microsphere is not a plasma membrane, thus the microsphere does not contain any ion transporter such as ion channel and sodium-potassium pump. Nevertheless, the microsphere generated a steady potential around the negative several tens millivolts (Ishima et al., 1981), which is almost the same as the resting potential of a living cell. In addition to that, the microsphere exhibited action potential-like potential spike, and its amplitude was some tens of millivolts which is the same as the amplitude of the action potential of a living cell (Ishima et al., 1981; Przybylski et al., 1982; Przybylski and Fox, 1984; Haefner, 1992). Now, one question arises because of such a finding: is the membrane potential generation a biological activity? It might be too early to raise such a question since no one has stated that the characteristics of the microsphere are similar to those of the living cell in all aspects. No one has investigated if the microsphere can exhibit all-or-none law, the refractory period, selective solute accumulation and exclusion, etc. which are the noticeable characteristics of the living cell. On top of that, the theoretical analysis of the microsphere using featuring physiological theories such as Goldman - Hodgkin - Katz equation (GHK eq.) and Hodgkin - Huxley model (HH model) has not been performed yet. However, even the above-described similarity of the microsphere potential to the membrane potential was one intense trigger for raising the above question.

The GHK eq. and the HH model are the fundamental concepts of electrophysiology (Ling, 1992; Ling, 2001; Kishimoto, 1968; Tasaki, 1982; Cronin, 1987; Ermentrout and Terman, 2010). There has been a notion that they are completely in line with the functionalities of ion channel and sodium-potassium pump. However, no one has provided an explicit mathematical correlation between GHK eq. and the HH model, For example, Tasaki states in his book to the effect that the GHK eq. is not in line with the characteristics of the action potential (Tasaki, 1982). Even under such circumstances, researchers appear to fully trust both GHK eq. and the HH model. Therefore, it might be appropriate to say that researchers use these concepts depending on the type of physiological questions (Ling, 1992; Ling, 2001; Kishimoto, 1968; Tasaki, 1982; Cronin, 1987; Ermentrout and Terman, 2010). More than 50 years ago, Kishimoto described that the HH model is not mathematically in line with the GHK eq. (Kishimoto, 1968), and he took up a simple example to explain it. This is described here with the authors' supplementary comment as follows: Eq. (1) is a typical equation of resting potential of a living cell, V, derived from the HH model, where g_j ($j = Na^+, K^+, Cl^-$) represents conductance and E_j represents the equilibrium potential. E_j is given by Eq. (2) where k, T, z_j, and e represent the Boltzmann constant, temperature, the valency of ion j and elementary charge, respectively.

$$V = \frac{1}{g_{Na} + g_K + g_{Cl}} \cdot [g_{Na}E_{Na} + g_K E_K + g_{Cl}E_{Cl}] \tag{1}$$

$$E_j = -\frac{kT}{z_j e} ln \left(\frac{[j]_{in}}{[j]_{out}} \right) \tag{2}$$

Eq. (3) is the well-known GHK eq. for the same system dealt by Eq. (1) where P_j represents permeability coefficient and $[j]_{in}$ and $[j]_{out}$ represent the concentration of ion, j, in the cell-interior and the cell-exterior, respectively.

$$V = -\frac{kT}{e} ln \frac{P_{Na}[Na^+]_{in} + P_K[K^+]_{in} + P_{Cl}[Cl^-]_{out}}{P_{Na}[Na^+]_{out} + P_K[K^+]_{out} + P_{Cl}[Cl^-]_{in}} \tag{3}$$

It is obvious that Eq. (3) cannot be derived using Eqs. (1) and (2). But Kishimoto neither denied the HH model nor the GHK eq. There appeared to exist a common notion in the physiology that then knowledge of HH model and GHK eq. was the right concepts but the contemporary knowledge of them had not been well-matured yet, therefore, those equations looked conflicting with each other. We feel that still there appears to exist such a notion even at present. Namely, the validity of this model and equations are not doubted but our understanding about them is doubted.

In this work, we scrutinized the physiological meaning of the HH model and GHK eq. to eradicate the mismatch between these concepts. The concepts were mathematically associated with each other by viewing the system in question as a thermodynamically real system rather than an ideal system. However, findings show that the broadly accepted membrane potential generation mechanism which attributes the nonzero membrane potential generation to the transmembrane ion transport was not tenable. Through the mathematical process of associating the GHK eq. and HH model, the authors concluded that the membrane potential generation could be due to the ion adsorption-desorption process in a thermodynamically real system rather than in a thermodynamically ideal system.

2. GHK eq. and HH eq.

2.1. Derivation of the GHK eq. using the ion adsorption mechanism

From our prior works, the GHK eq. could be reinterpreted as an inanimate model for predicting the potential generated by the electrolytic matters in an aqueous solution (Tamagawa and Ikeda, 2018; Tamagawa, 2019). In those works, the reinterpreted GHK eq. suggests that the membrane potential is not a consequence of the biological activity of the living cell but merely of the electrical potential generated by the ions distributed in the system in question by obeying thermodynamics.

The first step is to derive a formula for the electrical potential of the electrolytic matters in the aqueous solution systems such as the living cell, the microsphere of proteinoid, and the electrolytic hydrophilic polymers following our prior works (Tamagawa and Ikeda, 2018) and (Tamagawa, 2019). Since the actual living cell and the microsphere of proteinoid are complicatedly structured, a less complicatedly structured system is taken into consideration here as a simple cell model. The system focused on here is the mass of the hydrophilic polymer chains bearing carboxylic groups only. It is hypothesized that the polymer chains are equilibrated in a NaCl−KCl aqueous solution. Fig. 1 represents the "NaCl−KCl solution – polymer chain" system with a coordinate system, and this is the model that we will deal with.

Distribution of ion j in "NaCl−KCl solution – polymer chain" system is represented by Eq. (4) using "Boltzmann distribution" (Lewis and Randall, 1961), where the subscript p and s represent the polymer phase and the bathing solution phase, respectively. φ_p represents the polymer phase potential with respect to the bathing solution potential.

$$[j]_p = [j]_s \exp\left(-\frac{z_j e \varphi_p}{kT} \right) \tag{4}$$

The charge density of the polymer chain phase is given by Eq. (5).

$$\rho_p = e\Big([H^+]_p + [Na^+]_p + [K^+]_p - [OH^-]_p - [Cl^-]_p - [HCO_3^-]_p - [COO^-]_p\Big) \tag{5}$$

Eq. (5) is transformed into Eq. (6) using Eq. (4) where "A" and "B" are introduced in Eq. (6).

$$\rho_p = e\Bigg[\underbrace{([H^+]_s + [Na^+]_s + [K^+]_s)exp\Big(-\frac{e\varphi_p}{kT}\Big)}_{A} - \underbrace{([OH^-]_s + [Cl^-]_s + [HCO_3^-]_s)exp\Big(+\frac{e\varphi_p}{kT}\Big)}_{B} - [COO^-]_p\Bigg] = e\Big(A - B - [COO^-]_p\Big) \tag{6}$$

The total concentration of the carboxylic group is denoted by $[Carbo]$ as given by Eq. (7).

$$[Carbo] = [COO^-]_p + [COOH]_p + [COONa]_p + [COOK]_p \tag{7}$$

Eq. (8) is derived by "the mass action law" where K_j is association constant.

$$K_j = \frac{[COOj]_p}{[COO^-]_p[j]_p} \quad j = H^+,\ Na^+,\ K^+ \tag{8}$$

Eq. (9) is derived using Eqs. (7) and (8).

$$[COO^-]_p = \frac{[Carbo]}{1 + K_H[H^+]_p + K_{Na}[Na^+]_p + K_K[K^+]_p} \tag{9}$$

The bulk phase electroneutrality of the polymer chain phase is represented by Eq. (10) using Eq. (6).

$$[COO^-]_p = A - B \tag{10}$$

Eqs. (9) and (10) lead to Eq. (11).

$$K_H[H^+]_p + K_{Na}[Na^+]_p + K_K[K^+]_p = \frac{[Carbo] - (A - B)}{A - B} \tag{11}$$

Eq. (7) is also transformed into Eq. (12) using Eqs. (4), (8) and (10). The formula "_C" used to reach Eq. (12) is not needed. But it is introduced on purpose to explicitly show that the bulk phase "electroneutrality" represented by Eq. (10) is taken into consideration.

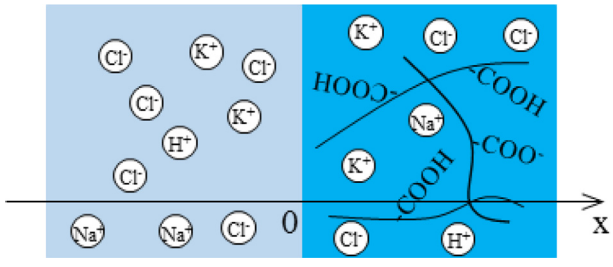

Fig. 1. Coordinate system set to the NaCl–KCl solution-polymer chain system.

$$\begin{aligned}&[COOH]_p + [COONa]_p + [COOK]_p \\ &\Big(= \underbrace{[Carbo] - [COO^-]_{p=Eq.10}}_{C}\ [Carbo] - (A - B)\Big) \\ &=_{Eq.8}[COO^-]_p(K_H[H^+]_p + K_{Na}[Na^+]_p + K_K[K^+]_p) \\ &=_{Eq.4}[COO^-]_p(K_H[H^+]_s + K_{Na}[Na^+]_s + K_K[K^+]_s)\ \exp\Big(-\frac{e\varphi_p}{kT}\Big)\end{aligned} \tag{12}$$

"The third line of Eq. (12) =The fourth line of Eq. (12)" is solved with respect to φ_p, resulting in Eq. (13).

$$\varphi_p = -\frac{kT}{e}ln\ \frac{K_H[H^+]_p + K_{Na}[Na^+]_p + K_K[K^+]_p}{K_H[H^+]_s + K_{Na}[Na^+]_s + K_K[K^+]_s} \tag{13}$$

Eq. (13) is the membrane potential formula for the system containing the three-dimensionally distributed ion adsorption sites (Tamagawa and Ikeda, 2018; Tamagawa, 2019). Although Eq. (13) is derived based on the ion adsorption concept, it is identical to the well-known GHK eq. (Ling, 1992; Ling, 2001). This result suggests that the potential formula identical to the GHK eq. is derivable even for a system without a continuous ion transport between the polymer chain phase and the bathing solution phase, as long as the system in question involves the ion adsorption-desorption process under the mass action law and the Boltzmann distribution.

G. N. Ling was the leading scientist who opposed the existing membrane potential generation mechanism, membrane theory (Ling, 1992; Ling, 2001). He suggested that the origin of the membrane potential lies in the ion adsorption-desorption process and not in the ion transport between the two phases. So, his theory is entirely in line with our suggestion that the potential formula can be derived by considering the ion adsorption-desorption process. Ling's theory significantly motivated this paper's work.

2.2. Membrane potential in an ideal or a real system

In this section, the derivation procedure of the membrane potential formula identical to Eq. (14) is scrutinized.

2.2.1. Membrane potential in an ideal system

Eq. (13) can be derived without following such a complicated procedure described in section 2.1. According to the Boltzmann distribution. Eq. (4) is derived.

Solving Eq. (14) with respect to φ_p results in Eq. (13).

$$\sum_{j=H^+,\ Na^+,\ K^+} K_j.[lhs\ of\ Eq.\ 4]_j = \sum_{j=H^+,\ Na^+,\ K^+} K_j.[rhs\ of\ Eq.\ 4]_j \tag{14}$$

So, what is needed to derive Eq. (13) is Boltzmann distribution only.

Similarly, solving Eq. (15) results in the ordinary GHK eq. such as Eq. (16).

$$\sum_{j=H^+,\ Na^+,\ K^+} P_j.[lhs\ of\ Eq.\ 4]_j = \sum_{j=H^+,\ Na^+,\ K^+} P_j.[rhs\ of\ Eq.\ 4]_j \tag{15}$$

$$\varphi_p = -\frac{kT}{e}ln\ \frac{P_H[H^+]_p + P_{Na}[Na^+]_p + P_K[K^+]_p}{P_H[H^+]_s + P_{Na}[Na^+]_s + P_K[K^+]_s} \tag{16}$$

So, the consideration of continuous ion transport between the NaCl–KCl solution and the polymer chain phase is not necessary for deriving the potential formula. It appears that the consideration of the adsorption-desorption process is not necessary, too. However, there should be a cause for realizing the heterogeneous ion

distribution which obeys the Boltzmann distribution, otherwise, the nonzero potential cannot be generated. The ion adsorption-desorption reaction inevitably takes place, as long as there exists a mobile ion and an adsorption site for it. Hence, it is not inappropriate to say that the ion adsorption-desorption process is a quite plausible reason for the membrane potential generation.

Eq. (4) suggests that the ion distribution governed by Boltzmann distribution and Eq. (14) is merely a linear combination of Eq. (4), and it is an identity equation of K_j, and similarly Eq. (15) should be an identity equation of P_j, too. Hence, any numerical value of K_j and P_j should make Eqs. (13) and (16) established, respectively. If Eqs. (13) and (16) do not hold, then Eqs. (14) and (15) do not hold. Therefore, Eq. (4) which is based on the fundamental thermodynamic concept of Boltzmann distribution becomes invalid. Eq. (16) is the GHK eq. but it is well-known that plugging only particular numerical values into P_j's of the GHK eq. can reproduce the experimental membrane potential (Ling, 1992; Ling, 2001). Hence, the actual GHK eq. is not an identity equation at all. It means that Eq. (16) is not an identity equation, and at the same time Eq. (13) is not an identity equation, either. It indicates the violation of the Boltzmann distribution. Now, one question about the GHK eq. could arise: Why is Eq. (13) (and Eq. (16)) out of the conceptual range of the Boltzmann distribution? This can be attributed to the conjecture one "The overlook of ion adsorption-desorption phenomenon" and the conjecture two "The system in question is a bit different from an ideal state." The conjecture one is commented on as follows: No matter what causes the membrane potential, Eq. (7) (the preserve of the quantity of carboxylic group) has to hold. Hence, Eq. (12) originating from Eq. (7) serves as a constraint for the electrical characteristics of the "NaCl—KCl solution – polymer chain" system. Therefore, K_j of Eq. (13) (which corresponds to P_j of Eq. (16)) cannot be arbitrary. The conjecture two is discussed in the next section.

2.2.2. GHK eq. for a real system

The ion distribution should obey the Boltzmann distribution. Hence, Eq. (4) should hold where $[j]_p$ and $[j]_s$ represent the ion concentration at the bulk phase of the polymer chain phase and the solution phase, respectively. But Eq. (4) is valid only when the system in question behaves ideally. Here, the ion distribution in the real system is discussed. It is known that the ion concentration of a real system, $[j]_q$ $(q = p, s)$ should not be expressed merely as the number of ions per unit volume, especially when the ion concentration is high. $[j]_q$ should be replaced by $\gamma_q^j[j]_q$ (see Eq. (17)) where γ_q^j is the activity coefficient often employed in physical chemistry (Lewis and Randall, 1961; Tamamushi and Goto, 1970; Sparks, 1984; Gimmi and Alt-Epping, 2018). It must be appropriate for the cell system to treat the ion distribution in such a manner since it is known that the cell characteristics do not necessarily behave ideally. For example, Ling introduced the finding by Höfler in his book the ref. (Ling, 1992). Höfler observed that the cytoplasmic part of the cell did not behave like a perfect osmometer. Ling and Negendank also suggested that a substantial part of the water in a frog's muscle is osmotically inactive (Ling, 1992).

$$[j]_q \rightarrow \gamma_q^j[j]_q \tag{17}$$

Using Eqs. (17) and (18), Eq. (16) turns into Eq. (19).

$$\gamma_q^j[j]_q \equiv a_q^j \tag{18}$$

$$\varphi_p = -\frac{kT}{e} ln \frac{P_H a_p^H + P_{Na} a_p^{Na} + P_K a_p^K}{P_H a_s^H + P_{Na} a_s^{Na} + P_K a_s^K} \tag{19}$$

Similarly, Eq. (13) should be represented by Eq. (20).

$$\varphi_p = -\frac{kT}{e} ln \frac{K_H a_p^H + K_{Na} a_p^{Na} + K_K a_p^K}{K_H a_s^H + K_{Na} a_s^{Na} + K_K a_s^K} \text{[Re—GHKeq.]} \tag{20}$$

In the previous section, this question was raised: "Why is Eq. (13) (and Eq. (16)) out of the conceptual range of the Boltzmann distribution?" and the answer is given as: the conjecture two "The system in question is a bit different from an ideal state." Additionally, the Re-GHK eq. (Eq. (20)) is a potential equation for the real system and it is slightly different from Eq. (13). Hence, Eq. (13) looks out of the conceptual range of the Boltzmann distribution. The same discussion is true for Eq. (16) as well.

2.3. Cause of membrane potential generation

Both Eqs. (19) and (20) are mathematically identical equations. The former equation is derived on the premise that the transmembrane ion transport is the cause of membrane potential generation, while the latter one is derived on the premise that the ion adsorption-desorption is responsible for the membrane potential generation (Ling, 1992). However, both Eqs. (19) and (20) are even derivable by using the concept of the Boltzmann distribution only. Hence, it is impossible at this moment to conclude on what is the right membrane potential generation mechanism. But we think that taking into consideration the ion adsorption-desorption process is fundamentally necessary to assess the potential quantitatively since the ion adsorption-desorption process inevitably takes place as described in section 2.2.1. On top of that, the ion adsorption-desorption mechanism excels the transmembrane ion transport mechanism as a cause of membrane potential because of the following reason: It is known that the plasma membrane is permeable to various ions, for instance, It is also known that the permeability coefficients of a living cell at the resting state is P_K: P_{Na}: $P_{Cl} = 1 : 0.04 : 0.45$ (Tasaki, 1982). G. N. Ling states in his book "… Cl^- is definitely not impermeant. Indeed, the high permeability of the red blood cell membrane to Cl^- has long been known…" (Ling, 1984). Nevertheless, it is also reported that the membrane potential is quite indifferent to the existence of Cl^- (Ling, 1992). This contradiction has not been settled yet, as long as the membrane potential generation is attributed to the transmembrane ion transport. On the other hand, the ion adsorption-desorption mechanism excludes the Cl^- influence on the membrane potential, since $-COO^-$ contained in a real living cell never binds to Cl^-. Of course, Cl^- can bind to $-NH_3^+$ fixed on the protein chain in a real living cell. Therefore, it may well be said that Cl^- must have some influence on the membrane potential, too. There is one plausible answer to this issue as follows: Three hydrogels, a cationic, an anionic and an amphoteric hydrogel, which contained amino groups, carboxylic groups, and both amino and carboxylic groups as immobile functional atomic groups, respectively, were synthesized in prior works of H.T. (Tamagawa, 1992). H.T. observed that amphoteric hydrogel volume change dependence on pH was quantitatively quite close to the volume change behavior of the anionic hydrogel rather than that of the cationic hydrogel. That is, the association and dissociation of the immobile amino group with a mobile anion are quite indifferent to the environmental ionic condition compared with the association and dissociation of an immobile carboxylic group with a mobile cation. Therefore, the same must be true for the protein. Hence, the influence of Cl^- on the membrane potential generation of a real living cell must be negligibly small. Therefore, Eq. (20) containing K_j rather than P_j is a quite plausible membrane potential mechanism.

H.T. previously derived a membrane potential formula, which is virtually the same as Eq. (13), as a right membrane potential

formula (Tamagawa and Ikeda, 2018; Tamagawa, 2019). Hence, the validation of Eq. (20) appears to invalidate the previous H.T.'s work. But such an issue is not a concern since Eq. (13) is approximately the same as Eq. (20) as long as the system in question is in the ideal state or is close to the ideal state.

2.4. Derivation of HH eq. from the GHK eq

Although both the GHK eq. and the HH eq. are regarded as fundamental physiological concepts, they have not been mathematically associated with each other yet (Kishimoto, 1968; Tasaki, 1982). Hence, the HH eq. is derived from the Re-GHK eq. of Eq. (20) here. The Re-GHK eq. is arranged into Eq. (21).

$$K_H a_p^H + K_{Na} a_p^{Na} + K_K a_p^K = \left(K_H a_s^H + K_{Na} a_s^{Na} + K_K a_s^K\right) exp\left(-\frac{e\varphi_p}{kT}\right) \tag{21}$$

Eq. (21) is decomposed into Eq. (22). Eq. (22) represents the Boltzmann distribution in a real system. Hence, it is thermodynamically valid.

$$\gamma_p^j [j]_p = \gamma_s^j [j]_s \exp\left(-\frac{e\varphi_p}{kT}\right) \tag{22}$$

Eq. (22) is solved with respect to φ_p, resulting in Eq. (23).

$$\varphi_p = -\frac{kT}{z_j e} ln\left(\frac{[j]_p}{[j]_s}.\frac{\gamma_p^j}{\gamma_s^j}\right) \leftrightarrow \varphi_p - \left[-\frac{kT}{z_j e} ln\left(\frac{[j]_p}{[j]_s}.\frac{\gamma_p^j}{\gamma_s^j}\right)\right] = 0 \tag{23}$$

Fig. 2 represents the circuit of the HH model (Kishimoto, 1968; Tasaki, 1982; Cronin, 1987; Ermentrout and Terman, 2010; HODGKIN and HUXLEY, 1952). So far, the ions taken into consideration are the cations only. But the typical HH eq. takes into consideration both the cations (K^+, Na^+) and the anion (Cl^-) (Ling, 1992; Ling, 2001; Kishimoto, 1968; Tasaki, 1982; Cronin, 1987; Ermentrout and Terman, 2010; HODGKIN and HUXLEY, 1952). Therefore, both the cations and the anion will be considered for the analysis to be described. Hence, I_ℓ in Fig. 2 is regarded as current by Cl^-, I_{Cl}. Thus, g_ℓ in Fig. 2 is regarded as g_{Cl}.

By the use of Eqs. (24) and (25), Eq. (23) is rewritten as Eq. (26) where $j = Na^+, K^+, Cl^-$.

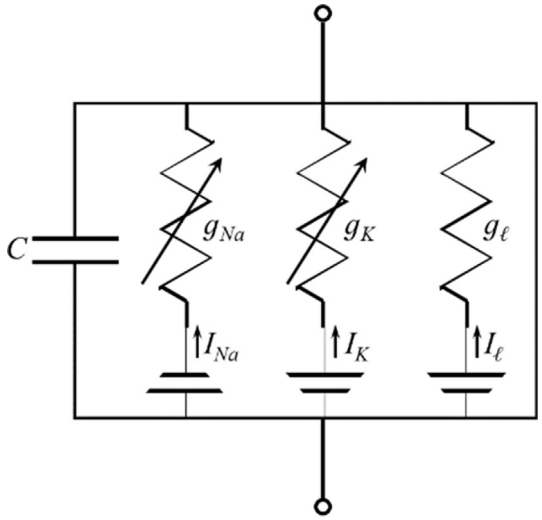

Fig. 2. Circuit of the HH model.

$$-\frac{kT}{z_j e} ln\left(\frac{[j]_p}{[j]_s}\right) \equiv E_j \tag{24}$$

$$-\frac{kT}{z_j e} ln\left(\frac{\gamma_p^j}{\gamma_s^j}\right) \equiv \Gamma_j \tag{25}$$

$$(\varphi_p - E_j) - \Gamma_j = 0 \tag{26}$$

Eq. (27) establishes regardless of the value of G_j ($j = Na^+, K^+, Cl^-$) where the meaning of G_j is discussed later on.

$$\sum_{j=\,Na^+,\,K^+,Cl^-} G_j.[lhs\ of\ Eq.\ 26]_j = \sum_{j=\,Na^+,\,K^+,Cl^-} G_j.[rhs\ of\ Eq.\ 26]_j \tag{27}$$

Eq. (28) is obtained by rearranging Eq. (27), and Eq. (28) is an identity equation of G_j.

$$G_{Na}(\varphi_p - E_{Na}) + G_K(\varphi_p - E_K) + G_{Cl}(\varphi_p - E_{Cl})$$

$$-G_{Na}\Gamma_{Na} - G_K\Gamma_K - G_{Cl}\Gamma_{Cl} = 0 \tag{28}$$

Eq. (28) is derived originally from Re-GHK eq. (Eq. (20)) and its physiological meaning is that the membrane potential is generated by the ion adsorption-desorption process. Now the resting potential and the action potential are analyzed using Eq. (28).

2.4.1. Resting state

The well-known HH eq. is often seen in textbooks and the resting potential formula in such textbooks is given by Eq. (29) where g_j is the conductance (Kishimoto, 1968; Tasaki, 1982; Cronin, 1987; Ermentrout and Terman, 2010; HODGKIN and HUXLEY, 1952). Bearing this equation in mind, Eq. (28) which is derived from Re-GHK eq. is to be discussed.

$$g_{Na}(\varphi_p - E_{Na}) + g_K(\varphi_p - E_K) + g_{Cl}(\varphi_p - E_{Cl}) = 0 \tag{29}$$

In the resting state, the activity coefficient γ_q^j in Γ_j of Eq. (28) takes certain constant values and it is denoted here by $\gamma_{q\_re}^j$. If Eq. (28) is identical to Eq. (29) which is based on the well-known HH model, Eqs. (30) and (31) have to hold.

$$G_j = g_j (j = Na^+,\ K^+,\ Cl^-) \tag{30}$$

$$G_{Na}\Gamma_{Na}^{re} + G_K\Gamma_K^{re} + G_{Cl}\Gamma_{Cl}^{re} = 0 \tag{31}$$

Is it possible to find the G_j ($= g_j$) sufficing Eqs. (28) and (31)? Yes, it is possible. Eq. (28) is an identity equation. Hence, any G_j is available. Therefore, what needs to be done is only to find the G_j sufficing Eq. (31).Γ_j^{re}

$$\Gamma_j^{re} \equiv -\frac{kT}{z_j e} ln\left(\frac{\gamma_{p\_re}^j}{\gamma_{s\_re}^j}\right) \tag{32}$$

Therefore, Eq. (29) is derivable from the Re-GHK eq. By introducing a new constant quantity $g_j = g_j^o$ which suffices Eq. (31), Eqs. (29) and (31) are rewritten by Eqs. (33) and (34), respectively, where φ_p^o represents the resting potential.

$$g_{Na}^o\left(\varphi_p^o - E_{Na}\right) + g_K^o\left(\varphi_p^o - E_K\right) + g_{Cl}^o\left(\varphi_p^o - E_{Cl}\right) = 0 \tag{33}$$

$$g_{Na}^o\Gamma_{Na}^{re} + g_K^o\Gamma_K^{re} + g_{Cl}^o\Gamma_{Cl}^{re} = 0 \tag{34}$$

Putting aside the physiological meaning of the g_j determined in such a manner, it is possible to mathematically associate the GHK eq. (Re-GHK eq. = Eq. (20)) with the HH model, as long as the system in question is a real system in the resting state.

2.4.2. Active state

The action potential generation mechanism using the Polarized Multilayer theory (PM theory) advocated by G. N. Ling is explained (Ling, 1992; Ling, 2001; Ling, 1978; Pollack, 2001). PM theory is not incorporated into the current physiology. Hence, the following proposal of action potential generation mechanism seems unlikely to be true, and one may think that some description is totally against the experimental evidence. However, the existing physiological concepts per se have various problematic facets. For example, the ion level disparity between cell-interior and cell-exterior is attributed by the functionalities of the ion channels and the pumps according to the current physiology, but it was reported by G. N. Ling that even dead cell exhibits such ion concentration disparity (Ling, 1992; Ling, 1978), and such a problem has remained unsolved up until today. Nevertheless, the ion level disparity is attributed to the metabolic activity of living cells in the mainstream physiology.

According to the PM theory, water molecules are adsorbed on the charged sites of polymer chains in the resting state as illustrated in Fig. 3(b). Another set of water molecules are adsorbed on these adsorbed water molecules owing to the dipole moment of water. This water adsorption process is repeated as in Fig. 3(c), resulting in a large scale structured water molecules occupying the whole system of the living cell, and ions are less soluble into the water contained in the cell compared to their solubility to the ordinary free water (Ling, 1992; Ling, 2001; Ling, 1978; Pollack, 2001). Therefore, the ions are largely in the adsorbed state to the immobile charges of the proteins, etc., which means γ_p^j is less than 1.

2.4.2.1. Potential Spike Induction Process

Fig. 4(a) is an illustration of a typical setup for measuring the potential of a living cell submerged in the electrolytic solution containing Na^+, K^+, and Cl^- where the reference potential is the external electrolytic solution potential. The cell contains charged polymer chains (proteins bearing immobile negatively charged carboxylic group and positively charged amino group) and negatively charged lipids (plasma membrane). These immobile positive charges can serve as adsorption sites for mobile anions, while these immobile negative charges can serve as adsorption sites for the mobile cations. Polymer chain in Fig. 4(a) bears six immobile positively charged adsorption sites and also six negatively charged adsorption sites. There are sixteen mobile cations and also sixteen mobile anions in the cell-interior, while there are ten mobile cations and ten mobile anions in the cell-exterior, too.

State (i): In the resting state (i) illustrated in Fig. 4(b), one Na^+, and three K^+ in the cell are adsorbed on four carboxyl groups, while five Cl^- are adsorbed on five amino groups. It is interpreted that four immobile negative charges out of the total six immobile negative charges are neutralized by the adsorption of four mobile cations, and five immobile charges out of the total six immobile positive charges are neutralized by five mobile cations. So, the net charge on the polymer chain is one negative charge. The other mobile Na^+ and K^+ in the internal cell phase are free or adsorbed on the negatively charged internal surface of the plasma membrane. A large quantity of Cl^- in the external electrolytic solution is in the free state, but on the other hand, a large quantity of Na^+ and K^+ are adsorbed on the external surface of the plasma membrane by their binding to the negative charges of the lipids. Water molecules in a real living cell are highly structured because of the immobile charges of proteins and lipids. Water molecules in the external solution are structured too but less structured due to the smaller quantity of proteins (smaller quantity of charge). The profile of the resting potential is indicated by (i) in Fig. 4(c).

State (ii): It is known that proteinous material exhibits thixotropy (Miura and Yamauchi, 1983), that is, an external force exerted on a gel material turns it into a sol. It can also be explained as the destruction of structured water by an external force. The destruction of structured water augments the quantity of free water, and it inevitably changes γ, since the ion's solubility to free water is different from its solubility to structured water. A similar phenomenon must take place when the external disturbance is exerted on a living cell which could result in the action potential generation as explained below:

Once some external disturbance is exerted to the system, the less structured water in the external solution becomes free first. Since free water has high solvency to ions compared to structured water (Ling, 1992; Ling, 2001; Ling, 1978; Pollack, 2001), Na^+ and K^+ adsorbed on the external surface of the plasma membrane are liberated as illustrated as "(ii) disturbed state" in Fig. 4(b). Therefore, the cell-exterior potential plunges due to the creation of immobile negative charges of lipids by the liberation (dissociation) of Na^+ and K^+ from lipids. Consequently, such a potential plunge results in soaring of the cell potential in reference to the cell-exterior. Its potential profile is indicated by (ii) in Fig. 4(c). One may argue that the cell-exterior charge soars due to the positive charges of the liberated Na^+ and K^+. However, the cell-exterior of the real system contains an immeasurable quantity of free mobile cations and anions which are in a thermal motion state. Therefore, the potential change by the increase of the free Na^+ and K^+ is nullified thermally. Hence, the potential soaring indicated by (ii) in Fig. 4(c) is observed.

State (iii): Immediately after State (ii), the relatively rigidly structured water molecules in the cell-interior collapses. Hence,

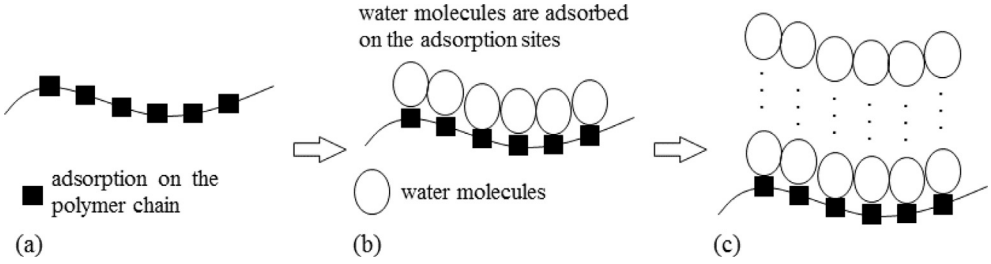

Fig. 3. Water structuring process on the polymer chain.

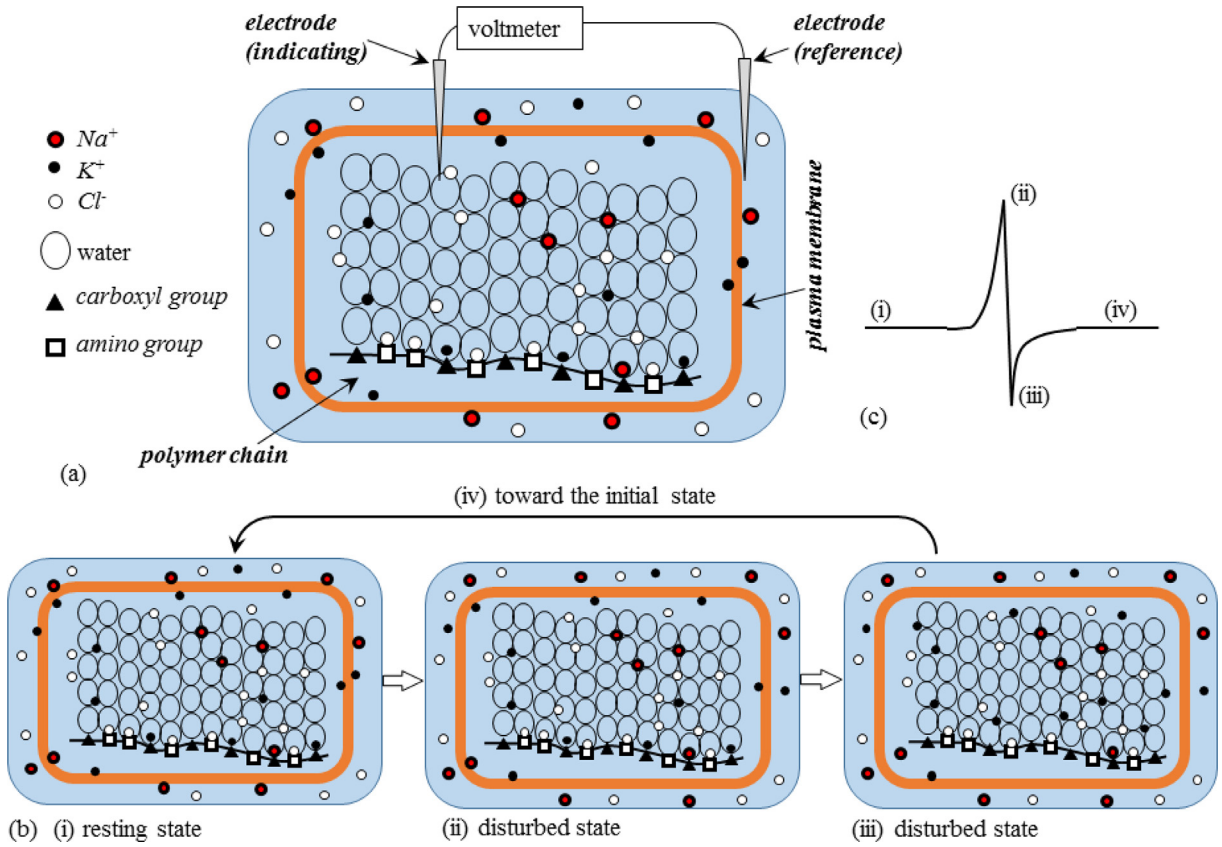

Fig. 4. Potential spike induction process (a) Experimental system (b) Nano level ion adsorption-desorption process (c) A typical behavior of action potential and a potential spike.

the large scale liberation of the adsorbed Na^+ and K^+ in the cell-interior is induced. But as to Cl^-, not a large quantity of Cl^- adsorbed on the amino groups are liberated as illustrated as "(iii) disturbed state" in Fig. 4(b) because the association-dissociation of Cl^- with an immobile amino group is not so sensitive to the external disturbance as described in section 2.3. Consequently, the polymer chain becomes negatively charged, resulting in the plunge of the cell-interior potential. Hence, the potential plunge indicated by (iii) in Fig. 4(c) takes place.

State (iv) = (i): Then, the system restores the initial state as indicated by "(iv)" in Fig. 4(b) and (c).

2.4.2.2. Potential Spike Induction Process and potential formula

Now, there is a need to verify if < Potential Spike Induction Process > is in line with Eq. (28). Eq. (28) is arranged into Eq. (35).

$$\varphi_p = \frac{1}{G_{Na}+G_K+G_{Cl}}\cdot[G_{Na}(E_{Na}+\Gamma_{Na})+G_K(E_K+\Gamma_K)+G_{Cl}(E_{Cl}+\Gamma_{Cl})]$$
$$= \frac{1}{g_{Na}+g_K+g_{Cl}}\cdot[g_{Na}E_{Na}+g_K E_K+g_{Cl}E_{Cl}$$
$$-\frac{kT}{e}ln\left(\frac{\gamma_p^{Na}}{\gamma_s^{Na}}\right)^{g_{Na}}\left(\frac{\gamma_p^{K}}{\gamma_s^{K}}\right)^{g_K}\left(\frac{\gamma_s^{Cl}}{\gamma_p^{Cl}}\right)^{g_{Cl}}] \tag{35}$$

g_j is merely an arbitrary coefficient and is defined by Eqs. (27) and (30). g_j^o is introduced when the system in question is in the resting state as given in Eqs. (33) and (34). In this work, we attribute the time-dependent potential behavior (action potential generation) to the time-dependent characteristics of the activity, $\gamma_q^j(t)$ not to g_j. Hence, we regard g_j in Eq. (35) as a constant represented by g_j^o even in the active state. The membrane potential is in principle expressed by Eq. (36) in this work. Bear in mind that Eq. (36) is originally derived from Re-GHK eq.

$$\varphi_p = \frac{1}{g_{Na}+g_K+g_{Cl}}\cdot[g_{Na}(E_{Na}+\Gamma_{Na})+g_K(E_K+\Gamma_K)+g_{Cl}(E_{Cl}+\Gamma_{Cl})]$$
$$= \frac{1}{g_{Na}^o+g_K^o+g_{Cl}^o}\cdot[g_{Na}^o E_{Na}+g_K^o E_K+g_{Cl}^o E_{Cl}$$
$$-\frac{kT}{e}ln\left(\frac{\gamma_p^{Na}(t)}{\gamma_s^{Na}(t)}\right)^{g_{Na}^o}\left(\frac{\gamma_p^{K}(t)}{\gamma_s^{K}(t)}\right)^{g_K^o}\left(\frac{\gamma_s^{Cl}(t)}{\gamma_p^{Cl}(t)}\right)^{g_{Cl}^o}] \tag{36}$$

State (i): In this state, the system is in the resting state. Hence, Eq. (34) holds. Using Eqs. (34) and (36), Eq. (37) is derived.

$$\left(\frac{\gamma_{p\_re}^{Na}}{\gamma_{s\_re}^{Na}}\right)^{g_{Na}^o}\left(\frac{\gamma_{p\_re}^{K}}{\gamma_{s\_re}^{K}}\right)^{g_K^o}\left(\frac{\gamma_{s\_re}^{Cl}}{\gamma_{p\_re}^{Cl}}\right)^{g_{Cl}^o} = 1 \tag{37}$$

The resting potential, φ_p^o, is given by Eq. (38) using Eqs. (36) and (37).

$$g_{Na}^o\left(\varphi_p^o - E_{Na}\right) + g_K^o\left(\varphi_p^o - E_K\right) + g_{Cl}^o\left(\varphi_p^o - E_{Cl}\right) = 0$$

$$\leftrightarrow \varphi_p^o = \frac{g_{Na}^o E_{Na} + g_K^o E_K + g_{Cl}^o E_{Cl}}{g_{Na}^o + g_K^o + g_{Cl}^o} \tag{38}$$

State (ii): The membrane potential is given by Eq. (36). This work hypothesizes that the membrane potential change is caused by the ion adsorption-desorption process and it is reflected in the ion activity change. In State (ii), the cell-exterior disturbance causes the liberation of the adsorbed Na^+ and K^+ from the external surface of the plasma membrane, that is, Na^+ and K^+ activities of cell-exterior increase. It can be reinterpreted that $\gamma_s^{Na}(t)$ increases from $\gamma_{s\_re}^{Na}$ $(=\gamma_s^{Na}(0))$ and $\gamma_s^K(t)$ increases from $\gamma_{s\_re}^K$ $(=\gamma_s^K(0))$ according to the formularization so far described. The increase in these quantities results in the raise of φ_p of Eq. (36). This explanation and the formulas are in line with the State (ii) described in < Potential Spike Induction Process >. However, the existing physiological theory states that the potential spike is caused by the influx of Na^+ across the plasma membrane (Ling, 1992). It could be interpreted that $\gamma_s^{Na}(t)$ decreases and $\gamma_p^{Na}(t)$ increases, while our theory suggests that $\gamma_s^{Na}(t)$ increases and $\gamma_p^{Na}(t)$ is kept constant. So, there appears to be a serious discord between physiological fact and Eq. (36). However, assuming that the Na^+ rushing into the cell-interior across the plasma membrane could be created due to the liberation of Na^+ from the external surface of the plasma membrane in view of the existing theory, the Na^+ activity of the cell-exterior must increase at least once even in view of the existing theory. The same must be true for K^+, too. Thus, the discord between physiological fact and Eq. (36) can be settled. Ions cannot flow independently, but they can only flow by maintaining electroneutrality which is a basic physical chemistry law. Hence, the cations and the anions are always nearby each other. Therefore, the ion quantity variation cannot be a cause of the potential change. In prior work of H.T., he observed that the ion concentration change did not induce any potential change unless there exist ion adsorption sites in the system (Tamagawa and Ikeda, 2017). H.T. supposed that the variation of the degree of ion adsorption can be a cause of potential change and therefore, the potential soaring is reflected as the increase of $\gamma_s^{Na}(t)$ and $\gamma_s^K(t)$ in this State (ii). Since the concentration of Na^+ at the cell-exterior is by far greater than that of K^+, primarily the increase of Na^+ quantity of the cell-exterior is responsible for the membrane potential soaring. Consequently, the prediction of Eq. (36) is basically in line with the existing physiological notion that the potential spike is caused by the influx of Na^+ across the plasma membrane.

State (iii): In this state, the structured water in the cell-interior collapses, and primarily Na^+ and K^+ in the cell-interior are liberated. It is interpreted that $\gamma_p^{Na}(t)$ increases from $\gamma_{p\_re}^{Na}$ $(=\gamma_p^{Na}(0))$ and $\gamma_p^K(t)$ increases from $\gamma_{p\_re}^K$ $(=\gamma_p^K(0))$. The increase in these quantities results in the plunge of φ_p of Eq. (36). This explanation is in line with the State (iii) described in < Potential Spike Induction Process >. However, the existing physiological theory states that the potential plunge is caused by the efflux of K^+ across the plasma membrane (Ling, 1992). It could be interpreted that $\gamma_p^K(t)$ decreases and $\gamma_s^K(t)$ increases, while our theory suggests that $\gamma_p^K(t)$ increases and $\gamma_s^K(t)$ is kept constant. So, there appears to be a serious discord between the existing theory and Eq. (36) again. But this issue can be settled in the same way described in State (ii) as follows: Assuming that the creation of K^+ coming out with rush from the cell-interior across the plasma membrane could be due to the liberation of K^+ of the cell-interior in view of the existing theory, $\gamma_p^K(t)$ must at least increase once before coming out with a rush from the cell-interior even in view of the existing theory. Therefore, the prediction of Eq. (36) is basically in line with the existing physiological notion that the potential plunge is caused by the efflux of K^+ across the plasma membrane.

State (iv) = (i): The initial state of the cell is restored, and which is interpreted that $\gamma_p^{Na}(t)$ and $\gamma_p^K(t)$ take

$\gamma_{p\_re}^{Na}$ $(=\gamma_p^{Na}(0))$ and $\gamma_{p\_re}^K$ $(=\gamma_p^K(0))$, respectively. This explanation is in line with the State (iv) described in < Potential Spike Induction Process >.

Eq. (36) is shown here again using Γ_j $(=\Gamma_j(t))$. Eq. (39) is another expression of Eq. (36) which is derived originally from the Re-GHK eq.

$$\begin{aligned} &g_{Na}^o[\varphi_p(t) - E_{Na}] + g_K^o[\varphi_p(t) - E_K] + g_{Cl}^o[\varphi_p(t) - E_{Cl}] \\ &-[g_{Na}^o \Gamma_{Na}(t) + g_K^o \Gamma_K(t) + g_{Cl}^o \Gamma_{Cl}(t)] = 0 \\ &\leftrightarrow_{Eq.26} \sum_{j=Na^+,\,K^+,Cl^-} g_j^o \Gamma_j(t) - \sum_{j=Na^+,\,K^+,Cl^-} g_j^o[\varphi_p(t) - E_j] = 0 \end{aligned} \tag{39}$$

The ordinary HH eq. will be discussed from here on. Eq. (40) is the widely known equation of the HH model. Bear in mind that Eq. (40) is not derived from the Re-GHK eq. but is given from the ordinary HH circuit model (Ling, 1992; Ling, 2001; Kishimoto, 1968; Tasaki, 1982; Cronin, 1987; Ermentrout and Terman, 2010; HODGKIN and HUXLEY, 1952). Hence, g_j in Eq. (40) is a time-dependent quantity unlike g_j in Eq. (39) where g_j in Eq. (39) is given by g_j^o.

$$\begin{aligned} &g_{Na}(t)[\varphi_p(t) - E_{Na}] + g_K(t)[\varphi_p(t) - E_K] + g_{Cl}(t)[\varphi_p(t) - E_{Cl}] \\ &- I(t) + C\frac{d\varphi_p(t)}{dt} \\ &= 0 \end{aligned} \tag{40}$$

$g_j(t)$ is a time-dependent conductance where $g_j(t)$ $(j = Na^+, K^+, Cl^-)$ is given by Eqs. A1 ~ A3 in Appendix A, respectively. These equations are introduced in various textbooks such as references, (Cronin, 1987) and (Ermentrout and Terman, 2010).

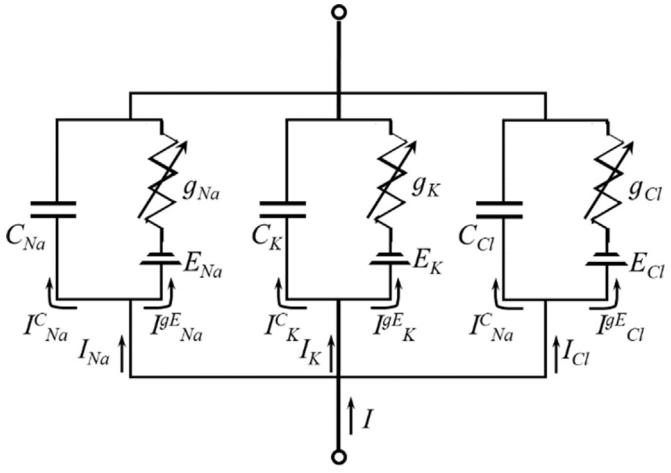

Fig. 5. Reconstructed HH circuit model.

Introducing Eq. (41), Eq. (40) is transformed into Eq. (42) where g_j^o in Eq. (41) has an identical quantity to g_j^o in Eq. (36).

$$g_j(t) = g_j^o + \Delta g_j(t) \tag{41}$$

$$\left[g_{Na}^o + \Delta g_{Na}(t)\right]\left[\varphi_p - E_{Na}\right] + \left[g_K^o + \Delta g_K(t)\right]\left[\varphi_p - E_K\right] + \left[g_{Cl}^o + \Delta g_{Cl}(t)\right]\left[\varphi_p - E_{Cl}\right]$$

$$-I(t) + C\frac{d\varphi_p(t)}{dt} = 0 \tag{42}$$

Now, it is possible to associate g_j^o and $\Delta g_j(t)$ with $\overline{g}_j$, m, h, and n as given in Appendix B (see Appendix A as to m, h, and n), namely, g_j^o is a given constant and $\Delta g_j(t)$ is a definable quantity to suffice Eq. (B1) ~ (B3). Bearing this fact in mind, further discussion is made.

Eq. (42) is derived by transforming the HH eq. of Eq. (40). It is further transformed into Eq. (43) using Eq. (26).

$$\left[g_{Na}^o + \Delta g_{Na}(t)\right]\Gamma_{Na}(t) + \left[g_K^o + \Delta g_K(t)\right]\Gamma_K(t) + \left[g_{Cl}^o + \Delta g_{Cl}(t)\right]\Gamma_{Cl}(t)$$

$$-I(t) + C\frac{d\varphi_p(t)}{dt} = 0 \tag{43}$$

Eq. (43) can be expressed by Eq. (44).

$$\sum_{j=\,Na^+,\,K^+,Cl^-}\left[g_j^o\Gamma_j(t) + \Delta g_j(t)\Gamma_j(t)\right] - I(t) + C\frac{d\varphi_p(t)}{dt} = 0 \tag{44}$$

Assuming that Eq. (39) is the same as Eq. (44), Eq. (45) is derived consequently.

$$\sum_{j=\,Na^+,\,K^+,Cl^-}\left[g_j^o\varphi_p(t) - g_j^oE_j + \Delta g_j(t)\Gamma_j(t)\right] = I(t) - C\frac{d\varphi_p(t)}{dt} \tag{45}$$

Assuming that Eqs. (46) and (47) are valid, Eq (48) is derived from Eq. (45).

$$C = \sum_{j=\,Na^+,\,K^+,Cl^-} C_j \tag{46}$$

$$I(t) = \sum_{j=\,Na^+,\,K^+,Cl^-} I_j(t) \tag{47}$$

$$\sum_{j=\,Na^+,\,K^+,Cl^-}\left[g_j^o\varphi_p(t) - g_j^oE_j + \Delta g_j(t)\Gamma_j(t)\right]$$

$$= \sum_{j=\,Na^+,\,K^+,Cl^-}\left[I_j(t) - C_j\frac{d\varphi_p(t)}{dt}\right] \tag{48}$$

By trial and error, the HH circuit model in Fig. 2 was reconstructed into the model illustrated in Fig. 5. Therefore, Eq. (49) is derived.

$$I_j(t) = I_j^C(t) + I_j^{gE}(t) \tag{49}$$

Eq. (48) is rewritten by Eq. (50) using Eq. (49).

$$\sum_{j=\,Na^+,\,K^+,Cl^-}\left[g_j^o\varphi_p(t) - g_j^oE_j + \Delta g_j(t)\Gamma_j(t)\right]$$

$$= \sum_{j=\,Na^+,\,K^+,Cl^-}\left[I_j^C(t) + I_j^{gE}(t) - C_j\frac{d\varphi_p(t)}{dt}\right] \tag{50}$$

Consequently, Eqs. (51) and (52) which are under the constraint of Eq. (49) were arrived at:

$$I_j^{gE}(t) = g_j^o\varphi_p(t) - g_j^oE_j + \Delta g_j(t)\Gamma_j(t) \tag{51}$$

$$I_j^C(t) = C_j\frac{d\varphi_p(t)}{dt} \tag{52}$$

Using Eqs. (49), (51) and (52), Eq. (53) is derived where $I\,(t = 0) = 0$, $\varphi_p\,(t = 0) = \varphi_p^o$, $d\varphi_p/dt|_{t=0} = 0$ and $\Delta g_j\,(t = 0) = 0$.

$$I_j(t) = g_j^o\varphi_p(t) - g_j^oE_j + \Delta g_j(t)\Gamma_j(t) + C_j\frac{d\varphi_p(t)}{dt} \tag{53}$$

Eq. (54) is derived from Eq. (53) using Eq. (46).

$$I(t) = \sum_{j=\,Na^+,\,K^+,Cl^-} I_j(t)$$

$$= \left[\sum_{j=\,Na^+,\,K^+,Cl^-} g_j^o\right]\varphi_p(t) + \sum_{j=\,Na^+,\,K^+,Cl^-}\left[-g_j^oE_j + \Delta g_j(t)\Gamma_j(t)\right]$$

$$+C\frac{d\varphi_p(t)}{dt} \tag{54}$$

So, the HH eq. is associable with the Re-GHK eq. and the electrical characteristic of the system, $\varphi_p(t)$ and $I(t)$, can be mathematically formulated as given by Eq. (54) using the time-dependent activity coefficients, $\Gamma_j(t)$. Next, the meaning of $g_j(t)$ is deduced. In the HH model, $g_j(t)$ represents plasma membrane conductance. But the discussion so far described suggests that $g_j(t)$ can be viewed as the time-dependent function of activity coefficients due to the following reasons: $g_j(t)$ is given by Eq. (41) which consists of two

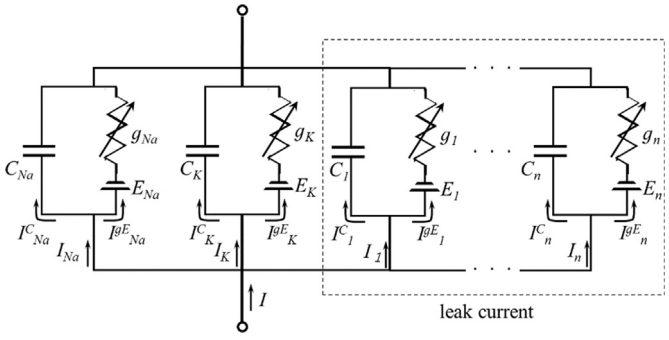

Fig. 6. General HH circuit model.

terms of g_j^o and $\Delta g_j(t)$. g_j^o is given by a function of activity coefficients which suffice Eq. (37). $\Delta g_j(t)$ can be expressed by a function of activity coefficients $\Gamma_j(t)$ which suffice Eq. (48). The molecular-level mechanism of the $\Delta g_j(t)$ behavior is still unknown. But the activity change by the ion adsorption-desorption can change the structure of the cell system and it could lead to the time-dependent electrical characteristics of $\Delta g_j(t)$, or it is even rather difficult to imagine that $\Delta g_j(t)$ is maintained constant even during the occurrence of ion adsorption-desorption.

2.5. A circuit model for treating multiple ionic species

In a real living cell system, multiple ionic species are involved in the membrane potential generation. Hence, it is quite natural to think that what is so far described takes into consideration only a small number of ionic species. One may argue that the leak current I_ℓ of Fig. 2 is not induced by Cl^- flow only. However, if so, the commonly known GHK eq. faces the same problem, since usually GHK eq. takes into consideration Na^+, K^+ and Cl^- only (Ling, 1992; Ling, 2001; Kishimoto, 1968; Tasaki, 1982; Cronin, 1987; Ermentrout and Terman, 2010; HODGKIN and HUXLEY, 1952). Here, we will attempt to deal with the multiple ionic species. Eq. (26) is valid for any mobile ion existing in the system in question. For instance, if Ca^{2+} is in the system, Eq. (26) holds by $j = Ca^{2+}$. Eq. (26) is originally Eq. (22). Eq. (22) is arranged into Eq. (55).

$$\left(\gamma_p^j[j]_p\right)^{1/z_j} = \left(\gamma_s^j[j]_s\right)^{1/z_j} \exp\left(-\frac{e\varphi_p}{kT}\right) \tag{55}$$

Eqs. (56) and (57) can be derived from Eqs. (18) and (55).

$$K_j\left(a_p^j\right)^{1/z_j} = K_j\left(a_s^j\right)^{1/z_j} \exp\left(-\frac{e\varphi_p}{kT}\right) \quad (j = \text{cation}) \tag{56}$$

$$K_j\left(a_s^j\right)^{-1/z_j} = K_j\left(a_p^j\right)^{-1/z_j} \exp\left(-\frac{e\varphi_p}{kT}\right) \quad (j = \text{anion}) \tag{57}$$

Solving Eq. (58) with respect to φ_p results in Eq. (59).

$$\sum_{j=\,all\ ions} [lhs\ of\ Eqs.\ 56, 57]_j = \sum_{j=\,all\ ions} [rhs\ of\ Eqs.\ 56,\ 57]_j \tag{58}$$

Eq. (59) is a general expression of Re-GHK eq.

$$\varphi_p = -\frac{kT}{e} ln\, \frac{\sum_{j=\,cations} K_j\left(a_p^j\right)^{1/z_j} + \sum_{j=\,anions} K_j\left(a_s^j\right)^{-1/z_j}}{\sum_{j=\,cations} K_j\left(a_s^j\right)^{1/z_j} + \sum_{j=\,anions} K_j\left(a_p^j\right)^{-1/z_j}} \tag{59}$$

Again, we pay attention to Eq. (26). The calculation of Eq. (60) results in Eq. (61), and it is the general formula of the HH model.

$$\sum_{j=\,all\ ions} G_j.[lhs\ of\ Eqs.\ 26]_j = \sum_{j=\,all\ ions} G_j.[rhs\ of\ Eqs.26]_j \tag{60}$$

$$\sum_{j=\,all\ ions} G_j(\varphi_p(t) - E_j) - \sum_{j=\,all\ ions} G_j\Gamma_j = 0 \tag{61}$$

Assuming Eqs. (62) and (63) for the resting state, the general expression of the resting potential is given by Eq. (64) by solving Eq. (60).

$$G_j = g_j^o \quad (j = all\ ions) \tag{62}$$

$$\sum_{j=\,all\ ions} G_j\Gamma_j^{re} = 0 \quad (j = all\ ions) \tag{63}$$

$$\varphi_p^o = \frac{\sum_{j=\,all\ ions} g_j^o E_j}{\sum_{j=\,all\ ions} g_j^o} \tag{64}$$

Next, the general expression of the action potential formula is to be derived from Eq. (26). By the same process of deriving Eq. (39), Eq. (65) is derived.

$$\sum_{j=\,all\ ions} g_j^o\left(\varphi_p(t) - E_j\right) - \sum_{j=\,all\ ions} g_j^o\Gamma_j = 0 \tag{65}$$

The circuit model for the general system should be represented by Fig. 6. For this circuit model, the commonly known HH eq. of Eq. (48) is generalized into Eq. (66), which is basically identical to Eq. (54), and Eq. (66) holds under the conditions given by Eqs. (49), (51) and (52) where $j = all\ ions$.

$$\sum_{j=\,all\ ions}\left[g_j^o\varphi_p(t) - g_j^o E_j + \Delta g_j(t)\Gamma_j(t)\right] = I(t) - C\frac{d\varphi_p(t)}{dt} \tag{66}$$

To sum up, GHK eq. and HH eq. for the system containing multiple ionic species can be attributed to one equation Eq. (26) which originates from the Re-GHK eq. Hence, even the general expressions of GHK eq. and HH eq. are mathematically associable with each other.

2.6. The physiological meaning of the HH model from the view of ion adsorption

The HH eq. and the GHK eq. (the Re-GHK eq.) are associable by viewing the system in question as a thermodynamically real system. However, the physiological interpretation of the circuit model of the HH eq. is not necessarily clear. This works points out that there still exists ambiguity in the meaning of the current HH model, since it can be formulated in another way using the thermodynamics of a real system. Here, a new quantity, the activity coefficient γ_q^j was imported into the HH model, but such a treatment may be quite artificial and inappropriate for the researchers who completely trust the HH model. However, without γ_q^j, the HH eq. becomes inconsistent with the GHK eq. (the Re-GHK eq.) and it has been a big issue remaining unsolved. In this work, it is supposed that the mathematical expressions of the HH eq. is merely another expression of the GHK eq. (the Re-GHK eq.) for a real system, but their physiological interpretation is a different story. Here, the circuit of Fig. 6 has not been physiologically reasoned out completely. For example, the current physiological meaning of the capacitor, C, of HH circuit model (Fig. 2) is at least insufficient from this paper's point of view as follows: C is regarded as the plasma membrane capacitor having a constant capacitance in the current HH model (Cronin, 1987; Ermentrout and Terman, 2010). However, it is strongly speculated that the actual capacitance changes when the cell is in the active state since the occurrence of transmembrane ion flow rate change is caused by a certain structural change of the plasma membrane. Therefore, forcibly regarding the variable of C as a constant C could inevitably cause the mismatch between the experimentally measured potential and the HH model-based potential. Nevertheless, the current HH model has worked successfully until today.

Concerning $\Delta g_j(t)$, its time-dependent characteristics are attributed to the ion transport across the plasma membrane in the current physiology (Andreoli et al., 1996; Tamagawa and Ikeda,

2017). However, this work suggests that it is also possible to attribute it to the ion adsorption-desorption process from the view of thermodynamics, too.

Through this work, it was found that it is necessary to view the living cell as a thermodynamically real system and it is also necessary to investigate the ion adsorption-desorption process as a cause of membrane potential for validating both the GHK eq. and the HH model and for mathematically associating with them each other.

A renowned physiologist, the late Tasaki had been active until he passed away. His work only a few years before his death was quite intriguing (Tasaki, 2005). Tasaki's work appears to suggest the need for ion adsorption-desorption process and the molecular structural change of cell body for the action potential induction and further indicates that the biological effect such as ion transporter-assisted transmembrane ion transport is not needed to induce the action potential. What is more, his work indicates that the action potential-like potential spike induction is not particular to the living cell. Tasaki's accomplishments appear to be well in line with the ion adsorption-desorption mechanism. Looking at the research field other than physiology, there have been a number of indications that the ion adsorption-desorption process could affect the membrane potential generation of a living cell. For example, the investigation of the electrical interaction between the charged matters in aqueous solutions is one of the primary research topics in the solution chemistry field and uncountable papers dealing with such a research topic have been continuously published (Watanabe and Kurihara, 1984; Israelachvili, 1985; Srinivasan et al., 1985; Brett and Brett, 1993; Bockris and Khan, 1993). However, physiologists have not been much aware of it even today. Moreover, the researchers out of physiological science have not been aware of the issues the authors have so far described.

3. Conclusion

It was demonstrated that it is possible to mathematically associate the HH model with the GHK eq. (the Re-GHK eq.) by viewing the cell as a real system. The Re-GHK eq. is based on the ion adsorption-desorption process. It means that the HH model can be reinterpreted from the view of the ion adsorption-desorption mechanism, although the appropriate physiological meaning to the circuit model of Fig. 5 was not given. At this moment, we cannot say that the currently accepted existing mechanism of membrane potential generation is wrong but think that the membrane potential mechanism is worth reinvestigating from the view of the ion adsorption mechanism.

CRediT authorship contribution statement

Hirohisa Tamagawa: Conceptualization, Methodology, Validation, Writing- Original draft preparation, Writing - review & editing. **Titus Mulembo:** Validation, Writing - review & editing. **Vera Maura Fernandes de Lima:** Validation, Visualization, Writing - review & editing. **Wolfgang Hanke:** Validation, Visualization, Writing - review & editing.

Appendix A

$$g_{Na}(t) = \overline{g}_{Na} m^3(\varphi_p,\ t) h(\varphi_p,\ t) \tag{A1}$$

$$g_K(t) = \overline{g}_K n^4(\varphi_p,\ t) \tag{A2}$$

$$g_{Cl}(t) = \overline{g}_{Cl} = const. \tag{A3}$$

m, h and n are given by Eqs. (A4)–(A6) according to the broadly accepted concept of HH model (Ling, 1984; Ling, 1978).

$$\frac{dm}{dt} = \alpha_m(\varphi_p)(1-m) - \beta_m(\varphi_p)m \tag{A4}$$

$$\frac{dh}{dt} = \alpha_h(\varphi_p)(1-h) - \beta_h(\varphi_p)h \tag{A5}$$

$$\frac{dn}{dt} = \alpha_n(\varphi_p)(1-n) - \beta_n(\varphi_p)n \tag{A6}$$

Appendix B

$$g^o_{Na} + \Delta g_{Na}(t) = \overline{g}_{Na} m^3(\varphi_p,\ t) h(\varphi_p,\ t) \tag{B1}$$

$$g^o_K + \Delta g_K(t) = \overline{g}_K n^4(\varphi_p,\ t) \tag{B2}$$

$$g^o_{Cl} + \Delta g_{Cl}(t) = \overline{g}_{Cl} \tag{B3}$$

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