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Isothermal Environmental Heat Energy Utilization by Transmembrane Electrostatically Localized Protons at the Liquid–Membrane Interface

James Weifu Lee*

ABSTRACT: This study employing the latest theory on transmembrane electrostatic proton localization has now, for the first time, consistently elucidated a decades-longstanding bioenergetic conundrum in alkalophilic bacteria and more importantly discovered an entirely new feature: isothermal environmental heat utilization by electrostatically localized protons at the liquid–membrane interface. It was surprisingly revealed that the protonic motive force (equivalent to Gibbs free energy) from the isothermal environmental heat energy utilization through the electrostatically localized protons is not constrained by the overall energetics of the redox-driven proton pump system because of the following: (a) the transmembrane electrostatically localized protons are not free to move away from the membrane surface as a protonic capacitor feature; (b) the proton pumps embedded in the cell membrane extend beyond the localized proton layer apparently as an asymmetric property of the biological membrane; and (c) the protonic inlet mouth of the ATP synthase that accepts protons is located within this layer as another natural property of the asymmetric biological membrane. This work has now, for the first time, shown a novel thermotrophic feature where biological systems can isothermally utilize environmental heat energy through transmembrane electrostatically localized protons to help drive ATP synthesis.

INTRODUCTION

Environmental heat is the dissipated form of heat energy, also known as latent (hidden) heat energy or the temperature-dependent molecular thermal motion energy in the environment. In the past, for centuries, it had been believed that environmental heat (the dissipated form of heat energy) in any mass medium could not be utilized to do useful work unless there is a temperature difference. That classic belief is now shattered by the new discovery reported here that transmembrane electrostatically localized excess protons at the liquid water–membrane interface can isothermally utilize their molecular thermal motions to do useful work in driving ATP synthesis at the biophysical molecular scale.

This is a groundbreaking scientific discovery also for the field of life sciences where it has been commonly believed that biological systems can use only chemical energy and/or light energy but not the environmental heat (the dissipated form of heat energy). Consequently, based on their sources of energy, the organisms on Earth were generally classified or believed only as two types: chemotrophs and phototrophs. This classic belief is now also shattered by the discovery reported here, which through a bioenergetics elucidation study of alkalophilic bacteria *Bacillus pseudofirmus* with the Lee transmembrane electrostatic proton localization theory surprisingly revealed that environmental heat can be isothermally utilized through electrostatically localized protons at a liquid–membrane interface to help drive ATP synthesis. Naturally, this raises an important scientific question: Do proton-coupling bioenergetic chemotrophs also have a thermotrophic feature? The answer to this question is now positive.

The story here began with the modern development of bioenergetics including Peter Mitchell’s chemiosmotic theory. Mitchell’s central bioenergetics equation has entered into many textbooks, which is expressed as the protonic motive force (pmf) across a biological membrane that drives protons through the ATP synthase; It is

\[
\text{pmf} = \Delta \psi = \frac{2.3 RT}{\mathcal{F}} \Delta \text{pH}
\]

Here, \(\Delta \psi\) is the electrical potential difference (which is defined as positive (p) phase minus negative (n) phase and is usually positive) across the membrane; \(R\) is the gas constant; \(T\) is the absolute temperature; \(F\) is Faraday’s constant; and \(\Delta \text{pH}\) is defined as the pH in the bulk p phase minus the pH in the bulk n phase. In that framework, the protons are
considered as solutes, like sugar molecules in water, that are delocalized, existing everywhere in the two bulk aqueous phases. Consequently, the Mitchellian view of bioenergetics is that the ATP synthase is coupled to the redox proton pumps via the bulk phase-to-bulk phase pmf across the biological membrane. The chemiosmotic theory was a significant milestone in the historical development of modern bioenergetics; its significance to the history of the field could hardly be overstated.

However, we now know that the textbook Mitchellian pmf equation (eq 1) is not entirely correct and thus should be revised.6,7,8,9,20.24 The well-established observations that disagree with the Mitchellian pmf equation are in alkalophilic bacteria, such as B. pseudoalcaliphilus.25-27 The alkalophilic bacteria keep their internal pH at about 2.3 units more acidic than the ambient bulk pH, while Δψ is about 180 mV.21-23 The use of the Mitchellian pmf equation (eq 1) in this case would yield a pmf value so small (44 mV at T = 298 K) that for decades has clearly failed to explain the energetics as to how these organisms are able to synthesize ATP.21-23

Furthermore, the classic Mitchellian chemiosmotic theory apparently has also failed to address the legitimate question as to what extent the proton-coupling pathway for producing ATP is delocalized throughout the bulk aqueous volume or localized at the membrane surface since it was first raised in 1961 by Williams.27-30.33 A number of well-documented observations, such as the localized proton-coupling characteristics observed in the "low salt"-treated thylakoids of the Chiang–Dilley experiment35 and the ΔpH surface component of pmf in ATP synthesis of mitochondria,36 could not be explained by the delocalized proton view of the classic Mitchellian chemiosmotic theory. The newly reported lateral pH gradient as elegantly probed with bioengineered fluorescent proteins as pH indicators between OXPHOS complex IV and F0F1 ATP-synthase in folded mitochondrial membranes also implicates a need for a "modification to Peter Mitchell’s chemiosmotic proposal." A recent biomimetic study37 points to a similar localized proton phenomenon.

Recently, as summarized briefly below, the author developed a transmembrane electrostatic protonation theory that elucidates how free excess protons in an aqueous medium separated by an impermeable membrane alone can be localized spontaneously at the liquid–membrane interface. Moreover, a newly developed pmf equation by modifying eq 1 to account for localized protons at a liquid–membrane interface was introduced and shown to result in a large enough pmf for the synthesis of ATP in alkalophilic bacteria.

Our previous study7,9 has clarified that neither the Gouy–Chapman theory41 nor the Debye length concept could be applied to estimate the thickness of the transmembrane electrostatically localized excess protons at the liquid–membrane interface here because the classic equations used in calculating the Debye length are defined only for charge-balanced solutions including 1:1 electrolyte (such as NaCl) solutions.42 Since the membrane is a type of insulator film (not an electrode), the excess proton layer at the water-membrane interface is likely to be a special monolayer (with a thickness probably about 1 nm) but definitely not an "electric double layer" as that of a typical electrode/electrolyte solution system as expected by the Gouy–Chapman theory.43 The current understanding of an excess proton monolayer is also consistent with the known "electric double layer" phenomenon since the excess proton layer can be understood as an extension from the secondary (proton) layer of the anode’s "electric double layer" around the proton-conductive water body surface. From here, we can also understand that the protons and ions of any permanent "electric double layer" formed as a result of membrane surface’s fixed charges (e.g., the negatively charged phosphate groups of phospholipids) that permanently attract ions are irrelevant to the membrane potential Δψ in the protonic bioenergetics. This understanding is consistent with the conclusion made in the previous study:43 – "membrane surface-fixed-charges-attracted protons are not relevant to the protonic motive force" that drives ATP synthesis.

As previously reported,7,9 we have recently demonstrated aprotic capacitor: the formation of a transmembrane electrostatically localized layer of excess protons at the water–membrane interface in biomimetic experiments using an anode water–membrane–water cathode system.9 The experimental demonstration of the excess anions–membrane-excess proton capacitor provided a proof-of-principle mimicking of an energized biological membrane system such as a mitochondrion in an energized resting state. Through an application of the Lee transmembrane electrostatic proton localization theory, the bioenergetic significance of mitochondrial cristae formation has now also been successfully elucidated.44

In this paper, the newly formulated pmf equation of refs 6, 9, and 40 is now extended to systematically calculate the energetics including the protonic motive force (pmf) and Gibbs free energy employing our experimentally measured proton–proton exchange equilibrium constants for sodium, potassium, and magnesium and using the existing experimental data of the alkalophilic bacterium B. pseudoalcaliphilus.25-27 Surprisingly, very large pmf values are found, which not only can well explain how these organisms are able to synthesize ATP24–26 but also significantly exceed the maximum pmf that could be supported by the respiratory redox-driven proton pump system. Remarkably
ably, as reported below, this subsequently resulted in a major new finding that the alkalophilic bacteria are not only chemotrophs but also have a thermotrophic feature that can create significant amounts of Gibbs free energy through isothermal utilization of environmental heat energy with electrostatically localized protons (protonic thermal motion kinetic energy) to do useful work such as driving ATP synthesis. Therefore, the study reported here through the energetic analysis of a well-defined biological system, alkalophilic bacterium, represents not only a significant progress in localized proton-coupling bioenergetics but also a major discovery for a novel biophysical energy transduction phenomenon: isothermal environmental heat energy utilization by transmembrane electrostatically localized protons at the liquid–membrane interface to do useful work such as driving ATP synthesis.

## RESULTS AND DISCUSSION

**Effect of Surface Localized Protons on the Protonic Motive Force.** The effect of surface localized protons on the protonic motive force (pmf) has now been analyzed using the newly formulated pmf equations (eqs 4–7) based on the transmembrane electrostatic proton localization theoretical framework (presented in the Materials and Methods section). Table 1 shows pmf values calculated from a set of the author’s newly formulated pmf equations (eqs 5–7) under the given *B. pseudoalteromonas* OF4 culture medium pH, cytoplasmic pH, and transmembrane electrical potential difference conditions and taking the effect of cation exchange into account. The calculated pmf as a function of the culture medium pH is also displayed in Figure 1, in comparison to the classic pmf (eq 1) and local pmf (the last term in eq 5) contributions. It is apparent from these results that the surface-localized protons dominantly contribute to the overall strength of the protonic motive force.

As listed in Table 1, at the culture medium pH (pHpB) of 7.5, the localized proton concentration [H+] at the liquid–membrane interface is as high as 1.28 × 10⁻² M. As the culture medium pH (pHpB) increased from 7.5 up to 11.4, the localized proton concentration [H+] decreased from 1.28 × 10⁻² M to as low as 6.21 × 10⁻¹³ M, primarily because the exchange reduction factor (the denominator in eq 7) increased from 1.50 to as big as 3.96 × 10¹¹ under the experimental conditions. That is, as the liquid culture medium pH (pHpB) increases, the thermodynamic equilibrium for the cation–proton exchange process appears to shift for more non-proton cations such as Na⁺, K⁺, and Mg²⁺ to occupy the liquid–membrane interface, thus reducing the localized proton concentration [H⁺]. However, even at the culture medium pH (pHpB) of 11.4, the localized proton concentration (6.21 × 10⁻¹¹ M) is still an order of magnitude higher than the culture medium bulk-phase proton concentration [H⁺B] of 3.98 × 10⁻¹² M.

Use of the localized proton concentration [H⁺] and the medium bulk-phase proton concentration [H⁺B] can now readily calculate the local pmf according to the third term of the author’s newly formulated protonic motive force (pmf) equation (eq 5). The values of the calculated local pmf are listed in Table 1 and plotted in Figure 1. As shown in Figure 1, the local pmf is typically much bigger than the classic pmf as calculated from the textbook Mitchellian pmf equation (eq 1). Therefore, the textbook Mitchellian pmf equation missed a major pmf component here.

The total pmf values, which equals to the sum of the local pmf and the classic pmf, are also listed in Table 1 and presented in Figure 1. As shown in Figure 1, the overall pattern of the total pmf amazingly matched with the *B. pseudoalteromonas* OF4 cell population growth observed experimentally with the cell population growth doubling times. That is, the pattern of the total pmf calculated through the newly formulated pmf equations (eqs 4–7) from the ground up using the real experimental data including the liquid culture medium pH (pHpB) and cation concentrations, the measured membrane potential (Δψ) and cytoplasmic pH (pHnB) excellently matched with the pattern of the observed cell population growth doubling times. This indicates that we really are on something real.

A pmf of at least 115 mV (equivalent to a Gibbs free energy of −11.1 kJ/mol) is needed to overcome the known phosphorylation potential (−497 mV at culture medium pH 10.5) for ATP synthesis with a proton-to-ATP ratio of 13/3 (497 mV/4.33 = 115 mV). The proton-to-ATP ratio of 13/3 is according to the known structures and functions of the *B. pseudoalteromonas* F₆F₄-ATP synthase, which has three catalytic sites for ATP synthesis, driven by a flow of 13 protons per revolution through the 13 c-subunits in its nanometer-scale molecular turbine ring.

As shown in Figure 1, the classic pmf such as 44 mV at liquid culture medium pH 10.5 is far below the minimally required pmf of 115 mV to drive ATP synthesis. Also, the pattern of the classic pmf does not match or make any sense with the observed cell population growth doubling times. For example, when the classic pmf goes up from 44 mV (at liquid culture medium pH 11.2) to 74 mV (at pH 11.4), one would expect that the cell population growth doubling times should go down because the increased pmf would support cell growth. However, the observed cell population growth doubling time went up (growth slower) instead of down. Neither the classic pmf value nor its pattern could explain the observed cell growth. This explains why the textbook Mitchellian pmf equation (eq 1) could never explain this bioenergetic problem, which was almost like an elephant in a room for the scientists who understood the issue.

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**Figure 1.** Calculated total, local, and classic pmf values of *B. pseudoalteromonas* OF4 as a function of external pH compared to the minimum value (115 mV) required to synthesize ATP and to the maximum possible value (redox potential energy pmf limit: 228 mV) that could be supported thermodynamically by the respiratory redox-driven proton pump system. The measured cell population growth doubling times are also superimposed. adapted with permission from ref 23. Copyright 2005 Elsevier
here over the last three decades in regard to how these microbes are able to synthesize ATP.24–26

On the other hand, the total pmf values including the contributions from the localized protons as shown in Figure 1 are well above the minimally required value of 115 mV in driving the synthesis of ATP, although the classic Mitchellian pmf is below this minimally required pmf for pH values at and above 8.5. This result can well explain why the B. pseudofirmus OF4 culture keeps such an excellent growth rate (cell doubling times less than 100 min) with culture pH ranging from 7.5 to 10.8. Furthermore, the decrease in total pmf when the liquid culture pH is raised beyond 10.8, owing to decreasing contributions from the localized protons caused by cation exchange, correlates amazingly well with the rapid increase in growth cell doubling times for pH values above 10.6. Particularly, the downward curve pattern of the newly calculated total pmf (as shown in Figure 1) matches excellently with the upward curve pattern of the experimentally measured cell doubling time in the entire range of the liquid cell culture medium pH from 7.5 to 11.4. Therefore, evidently, this result (data shown in Table 1 and Figure 1) from the use of the newly developed pmf expression in eqs 5–7 has now, for the first time, consistently provided an excellent elucidation for the 30 year longstanding energetic limit of the redox-driven proton pump system. This is also shown in Figure 1.

The maximum possible pmf value that could be supported by the respiratory redox-driven proton pump system, such as the one in B. pseudofirmus OF4, is only about 228 mV. The redox potential difference between the electron donor NADH (E_{m,NADH} = -320 mV) to the terminal electron acceptor O_{2} (E_{m,O2} = +820 mV) in this system is known to be about 1140 mV.16 For each pair of electrons from NADH to pass through the respiratory chain to the terminal electron acceptor O_{2}, the system drives the translocation of 10 protons across the membrane from the cytoplasm to the culture medium outside the cell. That is, it couples the translocation of 5 protons per electron across the membrane. Therefore, the maximum pmf that could be generated is about 228 mV per proton (1140 mV / 5 protons).

How is it possible that the total transmembrane pmf exceeds the limit imposed by the respiratory redox-driven proton pump system? We now understand here that the local pmf from localized protons is not constrained by the overall energetics limit of the redox-driven proton pump system and that the ambient environmental heat (representing the dissipated form of heat energy) in the cell culture medium is effectively utilized by the localized protons for ATP synthesis (driving the synthesis of ATP from ADP and Pi) through F_{F_{0}F_{1}}-ATP synthase, for the following reasons.

First, the localized protons are not free to move away from the membrane—liquid interface; they are electrostatically held at the membrane surface.19,20 Consequently, their thermal motion (environmental heat) will cause some to enter the protonic opening (F_{0} proton channel inlet) of the ATP synthase and be used to drive its nanometer-scale molecular turbine for synthesis of ATP from ADP and Pi.45,47,48

Secondly, the localized protons may not be directly coupled to the membrane-embedded redox proton pumps: namely, the respiratory chain complexes I, III, and IV.49 If they were, their ability to do work would be constrained by the energetics of the respiratory redox-driven proton pump system. A natural explanation of why this does not occur would be that the exit points for the translocated protons are outside of the surface layer of the electrostatically localized protons. Furthermore, to effectively make use of the localized proton thermal motion, the protonic entry point for the ATP synthase should be inside the localized proton surface layer. In this way, the redox-driven proton pump activity interacts with the proton activity in the bulk liquid phases but not with that of the localized proton layer at the liquid—membrane interface. Only the transmembrane electrical potential difference and the bulk-phase proton activity at the two sides of the membrane equilibrate with the proton-pumping respiratory electron-transport-chain activity. Consequently, the localized proton thermal motion provides additional free energy that may be utilized isothermally by the ATP synthase and is not limited by 228 mV.

The structures of bacterial respiratory membrane protein complexes, such as those in B. pseudofirmus OF4, are not well known. However, they are believed to be similar to the redox-driven proton-pumping complexes in mitochondria, which have been more extensively studied. Quite remarkably, the known structures of the mitochondrial respiratory protein complexes, as determined by cryo-electron microscopy and other molecular structural studies,49–57 fit amazingly well with this explanation. As shown in Figure 2, every one of the mitochondrial respiratory proton-pumping protein complexes I, III, and IV are seen to be all protruded by about 1 to 3 nm into the bulk liquid phase while the protonic inlet of ATP synthase (complex V) is located at the localized proton layer along the membrane surface. Adapted and modified with permission from ref 50. Copyright 2009 Elsevier.
corroborated with the known structures of the ATP synthase (complex V) and complexes I, II, III, and IV in the asymmetric biological membrane.

Apparently, the billion years of natural evolution already made the biological membrane asymmetric structures (Figure 2) perfect to constitute this amazing isothermal environmental heat utilization function with localized protons. This is also remarkably corroborated with our experimental observations that the localized protons at a liquid–membrane interface are "not detectable to a pH electrode in the bulk liquid phase." The biological systems apparently take advantage of this feature by "purposely" protruding the protonic outlets of their respiratory proton-pumping protein complexes I, III and IV into the bulk liquid phase to avoid the contact with localized protons.

Figure 3. (a) Proton-electrostatic localization model shown as "a proton capacitor-like structure" illustrating how excess protons (H\(^+\)) and hydroxyl ions (OH\(^-\)) are transmembrane electrostatically localized at the water–membrane interfaces along the two sides of a bacterial cell membrane before proton–cation exchange as it would be in an idealized pure water–membrane–water system; (b) protons can quickly translocate among water molecules by the "hops and turns" mechanism. Adapted with permission from ref 5. Copyright 2012 Creative Commons.
protons at the liquid—membrane interface while keeping the protons out of the ATP synthase precisely at the membrane surface to effectively utilize the localized protons that predominantly contribute to the total protonic motive force as shown by the data in Figure 1.

Therefore, we can now start to understand that the transmembrane electrostatically localized protons in combination with the asymmetric structural features of biological membrane especially in regard to the positions of the protonic pump outlets and the mouth of the localized proton users such as ATP synthase with respect to the localized proton layer along the p side of the membrane may constitute this special isothermal environmental heat energy utilization function. This is consistent with the understanding that the molecular thermal motion energy utilized isothermally as local pmf (equivalent to Gibbs free energy) is from the environment heat associated with the electrostatically localized protons. No new energy is created nor destroyed here. Therefore, this special isothermal environmental heat energy utilization function perfectly follows the first law (conservation of mass and energy) of thermodynamics but is not necessarily constrained by the redox-driven proton pump system that apparently well follows the second law of thermodynamics. Fundamentally, it is the transmembrane electrostatic proton localization with the effect of water as a protonic conductor (Figure 3) that enables the formation of a localized excess proton layer at the liquid—membrane interface over the mouths of the pmf users including the F0F1-ATP synthase (Figure 2). The formation of a localized excess proton layer at the water—membrane interface apparently results in some kind of “negative entropy effect” that brings the excess protons to the mouths of the pmf users where the protons can isothermally utilize their molecular thermal motions energy (proton thermal kinetic energy kBT) possibly including their Brownian motion to push through the doors of the protons to ATP synthase in driving ATP synthesis. Note, in the third term of eq 5 for the local pmf, the utilization of proton thermal kinetic energy kBT expressed as RT = kBTN, which equals to the product of the Boltzmann constant kB, the temperature T, and the Avogadro constant NA.

For the delocalized protons in the bulk liquid phase that are far away from the membrane surface, their thermal motions are not within the striking distance for them to hit into the F0F1-ATP synthase protonic channel to drive the rotary molecular machinery for ATP synthesis. Theoretically, to some extent, the delocalized protons could also do the work when they are at the liquid—membrane interface near the F0F1-ATP synthase. Therefore, the thermal energy factor RT = kBTN is also in the second term of the newly formulated pmf equation (eq 5). However, the value of log10[(H+]i/[H+]o)] was nearly zero in this case; consequently, the delocalized protons did not significantly contribute to the isothermal utilization of environmental heat in driving ATP synthesis here.

It is worth noting that the isothermal utilization of environmental heat (dissipated-heat energy) by transmembrane electrostatically localized protons at the liquid—membrane interface occurs without any physical phase transition such as the conventional liquid—gas phase change that would be required in the classic latent heat of water vaporization. Both the liquid phase inside the cell and the liquid culture medium outside the cell stay the same during the isothermal utilization of environmental heat through electrostatically localized protons.

Furthermore, there is no temperature difference across the biological membrane. Therefore, the utilization of dissipated-heat energy from the ambient temperature environment through transmembrane electrostatically localized protons at the liquid—membrane interface in driving ATP synthesis reported here is indeed a novel isothermal energy renewal process without involving any phase transition.

**Negative Gibbs Free Energy Change through Protonic Isothermal Environmental Heat Energy Utilization.** Note, the protonic motive force is equivalent to Gibbs free energy ΔG according to a simple relation with the Faraday constant (F) as in the following equation:

$$\Delta G = -F \times \text{pmf}$$  \hspace{1cm} (2)

Consequently, the amount of local pmf Gibbs free energy (ΔGL) resulting from this special isothermal environmental heat energy utilization function with localized protons can be calculated using the following equation:

$$\Delta G_L = -2.3RT \log_{10}(1 + [H^+]_L)/[H^+_{\text{gib}}])$$  \hspace{1cm} (3)

According to this equation (eq 3), as long as the localized proton concentration [H+]L is above zero, the Gibbs free energy (ΔGL) change is indeed a negative number. That is, the Gibbs free energy (ΔGL) change for the isothermal environmental heat utilization process is indeed negative if there is a localized proton concentration [H+]L (above zero). The amount of Gibbs free energy ΔGL created from the utilization of environmental heat through the localized protons is a function of the localized proton concentration [H+]L as shown in eq 3.

The proton-coupling bioenergetics systems widely operate in nearly all organisms known today. Therefore, through the present study as reported above, it is now quite clear that this special biological isothermal environmental heat utilization process associated with electrostatically localized protons has occurred probably for billions of years already on Earth. As shown in Figure 1, the amount of local pmf as calculated according to the third term of the newly formulated pmf equation (eq 5) quantitatively represents the activity of this amazing isothermal environmental heat (proton thermal kinetic energy kBT) utilization function with localized protons. Accordingly, it is the ratio of ([H+]L/[H+]o)]) of the localized proton concentration [H+]L at the membrane—liquid interface to the bulk-phase proton concentration [H+]o) at the same p side of the membrane that mathematically relates to this special isothermal environmental heat (protonic thermal kinetic energy kBT) utilization function, which is quite surprising.

The local pmf represents a very large portion of the total pmf. For example, when the liquid culture medium pH was 7.5, the local pmf (331 mV, equivalent to a Gibbs free energy of −32.0 kJ/mol) represents about 70% of the total pmf (471 mV, equivalent to a Gibbs free energy of −45.5 kJ/mol) as listed in Table 1. When the liquid culture medium pH was raised to 10.5, the local pmf (251 mV, equivalent to a Gibbs free energy of −24.2 kJ/mol) represents 85% of the total pmf (295 mV, equivalent to a Gibbs free energy of −28.5 kJ/mol). Since pmf is equivalent to Gibbs free energy ΔG according to eq 2, this result indicates that the values of Gibbs free energy ΔG created through isothermal utilization of environmental heat thermal energy by the localized protons are much more than the classic created free energy. That is, the bacteria obtain as much as 70−85% of the total pmf Gibbs free energy from the isothermal utilization of environmental heat energy associated with the localized protons. Therefore, it is now quite clear that
the bacteria have a significant thermotrophic property61 in addition to being chemotrophs.

In the past centuries, it was thought to be completely impossible to isothermally utilize the environmental heat “dead energy” dissipated in the ambient environment to do any useful work, which is also known as one of the classic second law statements.1,62,63 That classic belief is still largely true for many systems and processes around us including the cars and computers that we use. However, we now know, in certain special biophysical molecular systems such as the transmembrane electrostatically localized protons at the liquid–membrane interface, that classic belief may not necessarily be always true.

That is, the second law remains still as a very good law. However, it does not necessarily have to be always universal as implicated also by a number of independent studies.20,61,63

For example, many of the biological processes such as glycolysis, tricarboxylic acid cycle, and redox-driven proton transport apparently well follow the second law. Meanwhile, we now also understand, the special protonic isothermal environmental heat energy utilization function as reported here perfectly follows the first law (conservation of mass and energy) of thermodynamics but is not necessarily constrained by the redox-driven proton pump system that apparently well follows the second law of thermodynamics.

When the localized protons utilize environmental heat \( (k_B T) \) in helping drive the synthesis of ATP from ADP and Pi through the \( F_0 F_1 \)-ATP synthase (Figure 2), a fraction of the environmental heat \( (k_B T) \) energy may be locked into the chemical form of energy in ATP molecules; and it thus would result in a small drop in the environmental temperature theoretically because of the localized proton-associated isothermal environmental heat utilization. However, in the cell, there are many other processes (including the glycolysis, tricarboxylic acid cycle, and the redox-driven proton-pumping electron transport activities as well as the ATP utilization processes such as ATP hydrolysis) releasing heat energy, which could mask the isothermal environmental heat utilization process. That is, the energetic phenomenon of the cell may represent a complicated mixture of the chemotrophic processes and the isothermal environmental heat energy utilization process. This could probably also explain why it took so long for human beings on Earth to finally figure this out here.

Therefore, the discovery reported here may have profound scientific and practical implications in bettering the fundamental understanding of bioenergetics and energy renewal20 for sustainable development on Earth. For example, with the new knowledge learned from this discovery, it may be possible to have the benefits of mimicking this biophysical molecular scale process to create a new way20,61,71 in producing useful energy by isothermally utilizing the environmental heat energy from the ambient environment.

**CONCLUSIONS WITH THE DISCOVERY**

Taking transmembrane electrostatically localized protons into account with the newly developed expression of eqs 5–7 provides a large enough pmf to synthesize ATP in the \( B. pseudoflavus \) OF4 cells. Furthermore, owing to the reduction in localized protons due to increasing cation exchanges, the pmf decreases with increasing culture medium pH, well consistent with the observed decrease in bacteria growth rates. None of these features could be explained with the textbook Mitchellian pmf equation.10,74,75 It is remarkably shown here, for the first time, that the curve pattern of the newly calculated total pmf excellently matches with that of the experimentally measured cell doubling time, which now clearly elucidates the decades-longstanding energetic conundrum24–26 as to how the alkalophilic energetic bacteria are able to synthesize ATP and grow.

More importantly, we now understand with well-corroborated scientific evidence that biological membrane systems can isothermally utilize low-grade environmental heat energy associated with the ambient temperature thermal motion of transmembrane electrostatically localized protons (proton thermal kinetic energy \( k_B T \)) to perform useful work driving the synthesis of ATP. This amazing phenomenon can occur where the outlets of the redox-driven proton pumps are protruded away from the membrane surface to deliver protons into the bulk liquid phase, while the protonic inlet of the ATP synthase is located at the membrane surface. These features as predicted also match excellently with the true structures of mitochondrial respiratory membrane protein complexes I, II, III, and IV, and ATP synthase in relation to the location of the biological membrane surfaces.20,49–57

The Gibbs free energy \( (\Delta G_c) \) change for the isothermal environmental heat utilization process is indeed negative as long as the localized proton concentration \( [H^+] \) is above zero. The amount of Gibbs free energy \( \Delta G_c \) created from the utilization of environmental heat through the localized protons is a function of the localized proton concentration \( [H^+] \) as shown in eq 3. It is the ratio \( ([H^+]_L/\left[H^+_0\right]_L) \) of the localized proton concentration \( [H^+]_L \) at the membrane–liquid interface to the bulk-phase proton concentration \( [H^+_0] \) at the same p side of the membrane that mathematically relates to this special isothermal environmental heat (protonic thermal kinetic energy \( k_B T \)) utilization function.

Surprisingly, the local pmf represents a very large portion of the total pmf. That is, the bacteria obtain as much as 70–85% of the total pmf Gibbs free energy from the isothermal utilization of environmental heat energy associated with the localized protons. Consequently, the bacteria have a significant thermotrophic feature23 in addition to being chemotrophs.

Therefore, it is now quite clear that the pmf (equivalent to Gibbs free energy) from the isothermal utilization of environmental heat energy through the transmembrane electrostatically localized protons is not constrained by the overall energetics of the redox-driven proton pump system because of the following: (a) as a protonic capacitor feature, the localized protons are not free to move away from the membrane surface; (b) as an asymmetric property of the biological membrane, the proton pumps embedded in the cell membrane extend beyond the localized proton layer; and (c) as another natural property of the asymmetric biological membrane, the protonic inlet mouth of the ATP synthase that accepts protons is located within this localized proton layer. The biological systems can isothermally utilize the environmental heat energy through electrostatically localized protons to do useful work such as driving the synthesis of ATP. This is a major discovery that may have profound scientific implications in bettering the fundamental understanding of bioenergetics and isothermal environmental heat energy utilization in biological systems.

**MATERIALS AND METHODS WITH EQUATIONS**

Theoretical Framework for Protons Localized at Cell Membrane Surfaces. The basic process that leads to synthesis of ATP in cells and hence cell growth involves creating an excess number protons on one side of a biological membrane accompanied by a corresponding number of hydroxyl ions on
the other side, for instance, through the respiratory electron-transport-coupled proton pumps across the membrane. Subsequently, protons are transported to the protonic inlet of the ATP synthase complex embedded in the cell membrane.

Based on the transmembrane electrostatic proton localization theory,6,8,9,39 excess positively charged protons in an aqueous medium will electrostatically repel each other to become localized at the membrane surface on one side of a cell membrane, attracting an equal number of excess negatively charged hydroxyl anions to the other side of the membrane to form a “protonic capacitor” (Figure 3a). This theory is built on the premise that a liquid water body can act as a protonic conductor since protons can quickly transfer among water molecules by the “hops and turns” mechanism (Figure 3b) first outlined by Grøthuss.

As illustrated in Figure 3a and reported with great details in refs 6, 9, the theoretical support of the Lee transmembrane proton electrostatic localization model5,6,8,39 with a protonic capacitor system was first provided mathematically using the Gauss law integration equation of electrostatics with a differential surface element dS in polar coordinates r and R based on the premise of water as a protonic conductor and the fact that there can be zero electric field E inside a conductor. Indeed, such a protonic capacitor system with localized protons at a liquid—membrane interface has recently been verified experimentally in the Lee laboratory using a biomimetic water—membrane—water electrolysis system setup.7,19,20,39,58

Therefore, a generalized equation for the proton motive force (pmf) across a biological membrane taking into account the surface localized protons was introduced in refs 6, 9, 39 as

\[
\text{pmf} = \Delta \psi + \frac{2.3 RT}{F} \log_{10} \left( \frac{[H^+_\text{f}] + [H^+_\text{p}]/[H^-_{\text{p}b}]}{[H^+_\text{f}] + [H^+_\text{p}]/[H^-_{\text{p}b}]} \right) \tag{4}
\]

Here, \([H^+_\text{f}]\) is the concentration of the surface-localized protons, \([H^+_\text{p}]/[H^-_{\text{p}b}]\) is the proton concentration in the periplasmic bulk phase, and \([H^+_\text{p}]/[H^-_{\text{p}b}]\) is the proton concentration in the cytoplasmic bulk phase. Through algebraic rearrangement, this equation is now rewritten to isolate the contribution of the localized protons as follows:

\[
\text{pmf} = \Delta \psi + \frac{2.3 RT}{F} \log_{10} \left( \frac{[H^+_\text{f}]/[H^-_{\text{f}b}]}{1 + [H^+_\text{p}]/[H^-_{\text{p}b}]} \right) + \frac{2.3 RT}{F} \log_{10} \left( 1 + \frac{[H^+_\text{f}]/[H^-_{\text{f}b}]}{[H^+_\text{p}]/[H^-_{\text{p}b}]} \right) \tag{5}
\]

The first two terms of eq 5 comprise the Mitchellian “classic” bulk phase-to-bulk phase pmf, as noted in eq 1, whereas the last term accounts for the “local” pmf from the transmembrane electrostatically localized protons.

For an ideal protonic capacitor, the concentration of the ideal localized protons \([H^+_\text{f}]\) at the membrane—water interface is related to the transmembrane electrical potential difference \(\Delta \psi\) by

\[
[H^+_\text{f}]_0 = \frac{C}{S} \cdot \frac{\Delta \psi}{l} = \frac{\Delta \psi \cdot \varepsilon \cdot \varepsilon_o}{d \cdot l \cdot F} \tag{6}
\]

where \(C/S\) is the specific membrane capacitance per unit surface area, \(l\) is the thickness of the localized proton layer, \(\varepsilon\) is the dielectric constant of the membrane, \(\varepsilon_o\) is the electric permittivity, and \(d\) is the membrane thickness. In actual biological systems, non-proton cations in the aqueous media may exchange with protons localized at the membrane surface and thereby reduce their concentration. To account for this effect, we use

\[
[H^+_\text{f}] = \frac{[H^+_\text{f}]_0}{\prod_{i=1}^{n} \left( K_{pi} \frac{[M^+_{\text{ial}}]}{[H^+_\text{f}]} + 1 \right)} \tag{7}
\]

for the resulting concentration of localized surface protons. Here, \([M^+_{\text{ial}}]\) is the concentration of a given non-proton cation, and \(K_{pi}\) is the equilibrium constant for the cation to exchange with the localized protons.

It is noteworthy that all the physical quantities appearing in eqs 4—7 may, in principle, be determined experimentally with no freely adjustable parameters. For the membrane and proton layer parameters in eq 6, the calculations reported here have taken \(C/S = 13.2 \text{ mF/m}^2\) as an averaged membrane capacitance based on measured experimental data8 and \(l = 1 \text{ nm}\), which, as discussed in refs 6, 9, 19, is a reasonable thickness of the localized proton layer. Also, as discussed above, the localized proton layer thickness \(l\) may put constraints on the redox electron-transport-coupled proton pumps and proton gradient user structures that are embedded within a biological membrane.

In some of the literature, the membrane potential might somehow be reported as a “negative” number21—23 because of using an opposite reference orientation (from the cytoplasmic n side to the periplasmic p side)20,21 in contrast to that of the \(\Delta \psi\) defined in eqs 5 and 6 from the periplasmic p side to the cytoplasmic n side. In that case, special care should be taken to correct (remove) the negative sign for such membrane potential data reported with the opposite orientation such as that from ref 23 before applying it to the newly formulated pmf equation (eq 5) here.

In this study, the bioenergetics analysis of refs 6, 9 was extended by making a more complete treatment of the cation exchange using newly measured proton—cation exchange equilibrium constants for sodium and potassium cations55—58 to calculate the pmf and Gibbs free energy for the whole range of measured values for \(\Delta \psi\) \([H^+_\text{f}]/[H^-_{\text{f}b}]\), and \([H^+_\text{p}]/[H^-_{\text{p}b}]\), covering pH values between 7.5 to 11.5, for the well-documented \(B.\ pseudoaluminitis\) OF4 liquid culture experiments.21—23 These experimentally determined values are also shown in Figure 1. Also shown are the corresponding measured cell population growth doubling times, which were typically less than 100 min (excellent growth) in a culture medium pH \((\text{pH}_{\text{b}1})\) range from 7.5 to 10.8, except for its rapid increase (slower growth) for pH values between 10.8 and 11.5 (Figure 1). This work, as reported above, finally led to the discovery with well-corroborated evidence on an exciting new biological energy phenomenon: isothermal environmental heat energy utilization by transmembrane electrostatically localized protons at the liquid—membrane interface to do useful work such as driving ATP synthesis at the biophysical molecular scale.

**Effect of Cation Exchange with Transmembrane Electrostatically Localized Protons.** Concentrations of the cations Na+, K+, Mg2+, Ca2+, NH4+, Zn2+, Fe2+, Mn2+, Cu2+, and Co2+ in the liquid culture of \(B.\ pseudoaluminitis\) OF4 are presented in Table 2. They were calculated from the composition of the culture medium reported in refs 21, 82, 83. Also shown in Table 2 are the cation—cation exchange equilibrium constants \(K_{ji}\) used in this study. The \(K_{pi}\) values for Na+ and K+ were experimentally determined to be \(5.07 \times 10^{-8}\) and \(6.93 \times 10^{-8}\), respectively, through a study55 similar to that reported in ref 19. The average of these two values \((6.00 \times 10^{-8})\) was used to estimate \(K_{pi}\) for the other monovalent cation NH4+. The \(K_{pi}\) value of \(2.1 \times 10^{-7}\) for Mg2+ was calculated from experimental
data of cation exchange studies and was also used for the other divalent cations. Finally, Table 2 lists the calculated cation exchange reduction factors of the localized surface proton concentration for \( \text{pH}_{58} = 10.5 \). The total product of these factors (total cation exchange reduction factor in the denominator of eq 7) is a large number (44,100) compared to unity, indicating the very important role of cation–proton exchange at the membrane surface in controlling the localized surface proton concentration.

As reported previously in ref 6, the localized proton population density \( [\text{H}^+]_5 \) before the cation–proton exchange process was calculated to be 24.6 mM when the membrane electrical potential difference potential was 180 mV. In this example, the localized proton concentration \( [\text{H}^+]_5 \) is reduced apparently by the cation exchange to 24.6 mM/44100 = 5.58 \times 10^{-7} \text{ M}, which is equivalent to a localized proton \( \text{pH} \) of 6.25—remarkably lower than the bulk liquid culture \( \text{pH} \) of 10.5.

**ASSOCIATED CONTENT**

1. Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.0c01768.

Localized proton concentrations at the liquid–membrane interface, protonic motive forces, and Gibbs free energy (XLSX)

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J.W.L. designed and performed the research, analyzed the data, and wrote the paper.

**Notes**

The author declares no competing financial interest.

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