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Thermodynamic implications of high Q_{10} of thermo-TRP channels in living cells

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The activity of thermo-transient receptor potential (TRP) channels is highly dependent on temperature, and thus thermo-TRP reactions have a high temperature coefficient Q_{10}. In thermodynamics, a high value of Q_{10} indicates the existence of a large activation energy (i.e., a large enthalpy) over a short period during the transition process between the closed and open states of the channels. The Gibbs free energy equation shows that a large entropy is required to compensate for this large enthalpy and permit activation of the channels, suggesting a large conformational change of the channels. These large values of enthalpy and entropy seem to be a match for the values of the unfolding process of globular proteins. We outline these thermodynamic issues in thermo-TRPs.

Key words: enthalpy, entropy, Q_{10}, temperature dependency, TRP channel

Thermo-TRP channels

Other articles in this Special Issue will provide general information on transient receptor potential (TRP) channels. In the present study, we focus on so-called "thermo-TRPs", which are a subset of TRP channels. These channels are activated by distinct physiological temperatures, and are characterized by their unusually high temperature sensitivity ($Q_{10} > 0$; Q_{10} will be defined in the next section). TRPV1–V4 are activated by heat (>24°C), whereas TRPM8, TRPA1 and TRPC5 are activated by cold (<24°C). Although the values of Q_{10} for each channel have been reported to have a large variation [1–3], the table provided by Clapham and Miller is convenient as a standard reference (see Table 1 of the Clapham and Miller study) [4].

Structurally these thermo-TRPs are tetramers and each subunit contains six transmembrane domains (S1–S6), a hydrophobic pore loop linking transmembrane S5 and S6, and unusually large cytoplasmic *N*- and *C*-terminals (Fig. 1A). All thermo-TRPs have a variable number of ankyrin repeat domains in the *N*-terminus, except TRPM8, which has none and instead contains a TRPM homology region. Thermo-TRPs display distinct thermal thresholds from very noxious cold (TRPA1) to very harmful heat (TRPV2). Each thermo-TRP is also activated by specific natural or synthetic compounds that induce corresponding thermal and pain sensations in humans [5,6].

We still do not know exactly which part of a TRP channel senses temperature. However, previous studies strongly suggested that the *C* terminal plays an important role in activation mechanism by temperature change. (1) The deletion of some amino acids from the *C* terminal affected channel activation by heat [1]. (2) When the whole *C* terminal was switched between TRPV1 and the cold activated TRPM8, their temperature sensitivity properties were also swapped [3]. (3) The regions including the *C* terminal shifted the activation threshold temperature [7].

On the other hand, the binding sites with spider toxin and capsaicin, which result in activation of TRPV1, have been clarified based on structures [8,9] obtained using electron cryo-microscopy. From this information, one schematic presentation for the closed and open states of TRPV1 was sug-

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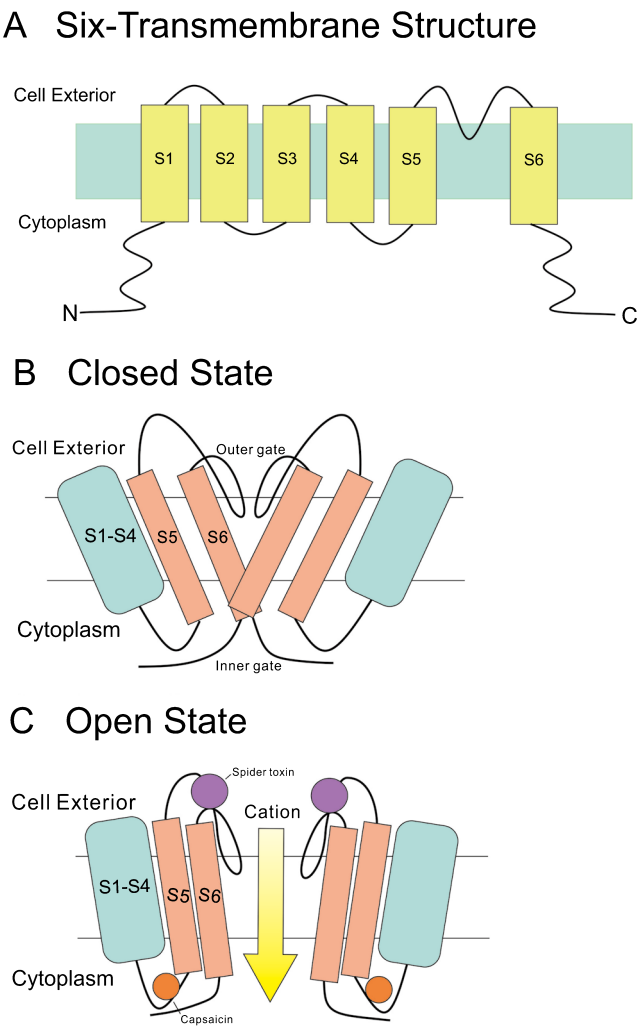

Figure 1 Structure of TRP channel. A. All TRP channels appear to have six transmembrane segments like the voltage-gated potassium channels. Both the *N* and *C* terminals are intracellularly located. The *C* terminal is considered to play an important role in the activation mechanism by heat. B. The ion selectivity filter region is identified from the linear sequence, as they resemble the ion selectivity filter of potassium channels. Two gates (outer gate and inner gate) are apparent in the closed state, one near the extracellular surface (outer gate) and another deeper within the channel (inner gate). C. The outer gate opens in response to the binding of spider toxin, whereas the inner gate opens on binding of capsaicin [10]. The arrow indicates the passage of cations, mainly Ca^{2+}, through the channel.

gested [10]. This scheme seems important for the activation by temperature change, too (Fig. 1B, C).

Temperature coefficient Q_{10}

The reaction rate in biological systems is temperature-dependent without exception. To model this dependency, the temperature coefficient (Q_{10}) is widely used; Q_{10} provides that the rate of a reaction increases for every 10-degree rise in the temperature. The definition of Q_{10} is given by the van't Hoff equation as follows:

$$Q_{10} = \left(\frac{k_2}{k_1}\right)^{\left(\frac{10}{T_2 - T_1}\right)}. \quad \text{(eq. 1)}$$

Here, k_1 is the reaction rate measured at T_1 degrees, and k_2 is the reaction rate measured at T_2 degrees. Note that the unit of T_1 and T_2 is Celsius or Kelvin and that T_1 and T_2 do not need to be exactly 10 degrees apart. If the reaction rate increases with increasing temperature, Q_{10} is greater than 1. For most mesophilic enzymes, Q_{10}=2–3 [11]. This shows that the rate of the reaction doubles or triples with every 10 K rise in temperature. The more temperature-dependent a process is, the higher is its Q_{10} value. If Q_{10}=1, the reaction can be explained by the diffusion of ions, and if Q_{10} values are greater than 2, the processes are thought to involve a large-scale protein conformational change, which will be discussed later.

Activation energy (enthalpy) corresponding to high Q_{10}

The Arrhenius equation is another formula used to present the temperature dependence of reaction rates. This equation is also based on the van't Hoff equation for the temperature dependence of equilibrium constants:

$$k = A \exp\left(-\frac{E_a}{RT}\right). \quad \text{(eq. 2)}$$

Here k is the reaction rate, A is the pre-exponential factor, E_a is the activation energy, R is the gas constant, and T is the temperature in Kelvin.

When the activation energies are obtained from two different temperatures, T_1 and T_2, with a difference of 10 degrees, we have:

$$T_1 = T, T_2 = T+10;\ k_1 = k_T, k_2 = k_{T+10}.$$

We calculate Q_{10} as

$$Q_{10} = \frac{k_{T+10}}{k_T} = \frac{A \exp\left\{-\frac{E_a}{R(T+10)}\right\}}{A \exp\left\{-\frac{E_a}{RT}\right\}}$$

$$= \exp\left[\frac{10E_a}{RT(T+10)}\right]. \quad \text{(eq. 3)}$$

Suppose two Q_{10} in which $Q_{10\alpha} > Q_{10\beta}$, then

$$\frac{Q_{10\alpha}}{Q_{10\beta}} = \exp\left[\frac{10(E_\alpha - E_\beta)}{RT(T+10)}\right], \quad \text{(eq. 4)}$$

and we obtain the relation between the two activation energies, $E_\alpha > E_\beta$. Therefore, the high Q_{10} value of thermo-TRPs corresponds to the large activation energy E_a. For example, if Q_{10}=20.0 within the temperature range of 303–313 K, we obtain E_a=226.4 (kJ mol^{-1}), and if Q_{10}=2.0 within the same temperature range, E_a=52.4 (kJ mol^{-1}). That is, when Q_{10} is ten-fold, the activation energy E_a is about four-fold.

For ion channels, Q_{10} can be measured from the whole-cell currents during temperature ramps, when the cells are maintained at a certain holding potential. For example, Voets *et al.* (2004) calculated from their own experimental data that E_a of the "opening" rate of TRPM8 was 15.7 kJ mol^{-1} and its corresponding Q_{10} was 1.2, whereas E_a of the "closing" rate of TRPM8 was 173 kJ mol^{-1} and its corresponding Q_{10} was 9.4 [12]. This result confirmed that a high Q_{10} value indicates a large activation energy E_a but showed that the barriers might be different between the opening process and the closing process.

The activation energy described above is equivalent to the "enthalpy", because all these values are derived from the same van't Hoff equation. Here, let us consider the slope of the van't Hoff plot for enthalpy. The change in the standard-state Gibbs free energy between 2 states (e.g., the open and closed states of channels) at a given temperature and pressure is expressed as

$$\Delta G^0 = -RT\ln k. \qquad \text{(eq. 5)}$$

Here, k is an equilibrium constant, corresponding to the reaction rate. Because the intrinsic difference in standard-state Gibbs free energy is

$$\Delta G^0 = \Delta H^0 - T\Delta S^0, \qquad \text{(eq. 6)}$$

the above two equations give

$$\ln k = -\frac{\Delta H^0}{RT} + \frac{\Delta S^0}{R}. \qquad \text{(eq. 7)}$$

When we use Equation 3 again for Q_{10},

$$Q_{10} = \frac{k_{T+10}}{k_T},$$

we can express this Q_{10} in a logarithmic form by use of Equation 7,

$$\ln Q_{10} = \ln k_{T+10} - \ln k_T = \frac{10\Delta H^0}{RT(T+10)}. \qquad \text{(eq. 8)}$$

Thus we obtain

$$\Delta H^0 = \frac{RT(T+10)\ln Q_{10}}{10}. \qquad \text{(eq. 9)}$$

Namely, we have the identical values for activation energy and enthalpy corresponding to the same Q_{10} in Equation 3. In some previous papers, the activation energy is referred to as the activation "enthalpy" [13].

Further, Equation 3 shows that Q_{10} is a function of temperature, so that when E_a is supposed to be constant, Q_{10} is higher in the low temperature range, whereas Q_{10} is lower in the high temperature range.

Entropy corresponding to high Q_{10}

To reduce ΔG^0, or to hold ΔG^0 to a small value, it is necessary that ΔS^0 of Equation 6 be sufficiently large [14]. We calculate that $\Delta S^0 = 0.723$ (kJ mol^{-1} K^{-1}) in the case of $\Delta H^0 = 226.4$ (kJ mol^{-1}) and $T = 313$ (K), in order to obtain $\Delta G^0 = 0$ (kJ mol^{-1}) at $Q_{10} = 20.0$. Indeed, such a compensation of ΔH^0 and ΔS^0 was confirmed in experiments for several different thermo-TRPs, most of which were performed by the Voets group [12,15–20]. In brief, these large values of enthalpy and entropy appear to be comparable with the values for "unfolding" of globular proteins (see Table 1 of the Robertson and Murphy study) [21].

As seen above, some thermo-TRPs with high Q_{10} require very large enthalpies and entropies. We now express enthalpy and entropy by the use of heat capacity [14], because a conformational change in proteins is thought to be accompanied with a change in heat capacity.

$$\Delta H^0(T) = \Delta H^0(T_{ref}) + \int_{T_{ref}}^{T} \Delta C_p \mathrm{d}T$$
$$= \Delta H^0(T_{ref}) + \Delta C_p(T - T_{ref}) \qquad \text{(eq. 10)}$$

$$\Delta S^0(T) = \Delta S^0(T_{ref}) + \int_{T_{ref}}^{T} \frac{\Delta C_p}{T} \mathrm{d}T$$
$$= \Delta S^0(T_{ref}) + \Delta C_p \ln\left(\frac{T}{T_{ref}}\right) \qquad \text{(eq. 11)}$$

Here, ΔC_p is a molar heat capacity and independent of T, and T_{ref} is a reference temperature chosen arbitrarily (e.g., standard ambient temperature, 298.15 K). Now we can substitute Equations 10 and 11 into Equation 7:

$$\ln k(T) = \ln k(T_{ref}) - \frac{\Delta C_p}{R}(1 - \ln T_{ref}) + \frac{\Delta C_p}{RT}(T\ln T + T_{ref})$$
$$= \frac{\Delta C_p}{R}\left(\ln T + \frac{T_{ref}}{T}\right) + constant. \qquad \text{(eq. 12)}$$

This Equation 12 demonstrates a U-shaped curve (but almost flat) when $0 < T < 2T_{ref}$, and it takes the minimum value at $T = T_{ref}$, because the first derivative $\frac{d\ln k(T)}{dT} = \frac{\Delta C_p}{RT^2}(T - T_{ref}) = 0$ at $T = T_{ref}$. When ΔC_p increases, the curvature of the U shape becomes acute.

Conformational changes of thermo-TRPs with high Q_{10}

Clapham and Miller (2011) remarked that, in Equation 12, the value of ΔC_p tends to be large and the curve tends to be U-shaped, and their analysis of this phenomenon deepened the understanding of thermo-TRPs underlying the temperature sensing [4]. If a TRP domain containing buried hydrophobic groups is thrust into a solvent upon channel opening, C_p is thought to increase. The conclusion from their theoretical analysis is as follows: "if 10–20 side chains in a TRP subunit—or indeed lipid moieties, which are, after all, part of the thermodynamic system—become exposed to water upon channel opening, the properties discussed here must emerge. They are necessary, direct consequences of the First and Second Laws of thermodynamics. Adding more states to

account for what is undoubtedly a multistep process does not alter this argument. As long as the function of hot- and cold-sensing TRPs involves the exposure of ≈20 nonpolar residues upon gating, then thermodynamics requires that a channel activated by high temperatures will also be activated [by] low temperatures (and vice versa)." If this scenario is true, the activation process of some thermo-TRPs resembles the "unfolding" or "denaturation" process of globular proteins [22].

More recently, Chowdhury and colleagues employed a protein engineering approach and the ΔC_p ideas to design a temperature-sensitive channel. They built thermosensitivity "bottom-up" into Shaker-like K^+ channels belonging to the same superfamily as TRPs, whose normal voltage-driven activation process is nearly temperature-independent [23]. They showed that the specific heat capacity change during channel gating is a major determinant of thermosensitive gating.

On the other hand, Jara-Oseguera and Islas (2013) have proposed an allosteric model (8-state model) that determines the temperature-dependent activity of thermo-TRPs, and have claimed that the properties in response to both cooling and heating can be explained in a "ΔC_p-independent" manner [24]. They showed that the activation of cold-activated channels can be achieved by a heat-activated temperature sensor that is characterized by positive ΔH^0 and ΔS^0 values. This fact is consistent with the notion that the activation by elevated temperature is accompanied by significant hysteresis, at least in the TRPV1, TRPV2 and TRPV3 channels [25–27]. These ideas are also supported by the similarity to protein folding/unfolding processes, as described in the following section.

They also agreed that the channel activity of thermo-TRPs shares some properties with folding/unfolding processes of proteins [25,27]. For example, the exposure of buried residues to the solvent and the breaking of some intermolecular interactions may occur in TRP channel activation in response to a change in temperature that can be observed as the molecular events accompanying a protein unfolding reaction. However, from the viewpoint that the channel activation is "ΔC_p-independent" (but the protein thermal denaturation is usually associated with large changes in heat capacity), Jara-Oseguera and Islas claimed that a small conformational change involving no more than 50 residues, which was hypothesized by Clapham and Miller [4], does not have to be accompanied by a large change in heat capacity [24]. Jara-Oseguera and Islas described [24] that "the exposure of hydrophobic protein regions to water that results in an increase in heat capacity could very well be balanced by the exposure of polar protein regions to the solvent, which reduces heat capacity [28]."

De novo structure control mechanism of TRP channels

Much progress has been made in the structural analysis of channel pores in relation to thermo-TRPs [8,12,20,29]. Recently, the Yamashita group investigated a fungal TRP channel, TRPGz, and reported that the structure of a cytosolic *C*-terminal domain, which is required to activate hyperosmosis and temperature increase, is a four-α helix bundle assembled in an offset spiral, with weaker interprotomer interactions [30]. This bundle structure is not always formed, and even when formed can easily come apart. When this bundle is formed, TRGz opens the channel in response to a temperature increase. On the other hand, TRP channels are inactivated by the binding with phosphatidylinositol 4,5-bisphosphate (PIP_2) [31,32], which has 5 to 6 negative charges from three PO_4 groups. It is only after the above-described cytosolic *C*-terminal domain bundle comes completely apart that the interaction between the basic cluster of the cytosolic *C*-terminal domain and the PIPs (including PIP_2) on the vacuolar membrane (organelle in animal cells) inhibits the channel activity, showing that the binding and disjunction of this four-α helix bundle plays an important role in the opening and closing processes of TRP channels. This phenomenon may be due to Coulomb interaction. This binding and disjunction of a bundle may also explain the findings of Voets et al.that the values of Q_{10} and E_a differed between the opening rate and the closing rate [12].

Perspective: Involvement of a change in vicinal water of the cytosol in hyperthermia cancer treatment

Recently, Mentré proposed a novel theory about the role of water in the cell, based on numerous results obtained in the field of physical chemistry [33]. Mentré maintains that misinterpretations of electron microscopy images have led researchers to completely overlook the existence of interfacial bound water. The interfacial water is the strongly constrained water surrounding proteins (i.e., bound water). The highly heterogeneous structure of this water layer reflects the heterogeneity of the protein surface. The interfacial water presents a large range of densities. Therefore, the changes in protein configuration must result in changes in the volume of the bound water, and thereby in the mechanical effects of the bound water. Mentré further avers that the whole cell water (70–80% of total mass) is distributed into only two to three hydration layers around proteins or macromolecules. Consequently, the average thickness of the space between the proteins in cells is estimated as only 1.2 nm [34]. In addition, the mean distance between two free Ca^{2+} ions is calculated as 55 nm at the concentration of 10 μM in cells [34]. Because the mean size of globular proteins is estimated as 3–5 nm, these Ca^{2+} ions are separated by tens of, or even hundreds of, proteins. If so, many previous analyses of electrophysiological data would have to be changed, because ions cannot

move by diffusion process in living cells.

Mentré's theory could offer great insight into the further development of cancer treatments. The classical concept of the Hofmeister series showed that Na^+ is naturally excluded from the interfacial water (i.e., bound water), but K^+ is little or not excluded from the interfacial water [35–37]. However, the vicinal water surrounding proteins in cancer cells is changed to free water (i.e., bulk water) and releases K^+, resulting in an increase in intracellular K^+ concentration. This result was confirmed by NMR experiments [38]. In other words, the water in cancer cells is thought to be easily moved via diffusion. Therefore, many of the results obtained so far by using cancer cell lines must be carefully revisited in studies using normal cells. Further, such differences between cancer cells and normal cells may explain why "hyperthermia treatment induced at 43°C" can selectively kill cancer cells even if normal and cancer cells are equipped with the same kinds of thermo-TRPs. Thermo-TRPs may play an important role in cancer treatment. The details for this hyperthermia mechanism will be discussed in the near future.

In conclusion, a high Q_{10} of thermo-TRPs requires both large enthalpy and large entropy values. This indicates that the activation process of the thermo-TRPs resembles an unfolding process of globular proteins. The structure of the water surrounding thermo-TRPs is expected to change in response to the change in the structure of thermo-TRPs. The contribution of the entropy term, as well as the enthalpy term, to ion permeation barriers has also been observed in another channel (K^+ channel) by molecular dynamics simulations [39].

Conflict of Interest

All the authors have no conflict of interest to report.

Author Contributions

E. I. and T. Y. directed the entire project. E. I. and Y. I. calculated the data. E. I., Y. I. and Y. T. co-wrote the manuscript.

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