We are IntechOpen,
the world's leading publisher of
Open Access books
Built by scientists, for scientists

6,900
Open access books available

185,000
International authors and editors

200M
Downloads

Our authors are among the

154
Countries delivered to

TOP 1%
most cited scientists

12.2%
Contributors from top 500 universities

CLARIVATE ANALYTICS
BOOK CITATION INDEX
INDEXED

WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com

Functional Difference Between Deuterated and Protonated Macromolecules

Takashi Sugiyama and Tohru Yoshioka
Kaohsiung Medical University,
Taiwan

1. Introduction

Water (H_2O) is very important to living materials, but it is a very strange liquid in the physical sense, because it has an unexpectedly high melting point (0 °C), boiling point (100 °C), density (0.998 g/cm^3), heat of vaporization (10,515 cal/mol), specific heat capacity and temperature of maximum density (4 °C), compared with other typical hydrogen compounds, such as H_2S, H_2Se and H_2Te. Furthermore, it has higher conductivity of proton than that of electron.

To make a water molecule, one pair of bonding hydrogen lose electrons and give them to electro-negative oxygen, and then each hydrogen will have a charge of +0.17e and one pair of electrons near oxygen will give 2x(-0.17e), this asymmetry of the water molecule leads to a dipole moment. The system of hydrogen bond in water determines the key to its biologically significant properties in forming a water network on the surface of proteins.

What is expected when hydrogen (H) is replaced by deuterium (D)? Heavy water (D_2O) has completely the same chemical characteristics as H_2O, but it is quite different in physical aspects, such as melting point (3.82 °C), boiling point (101.72 °C), density (1.017 g/cm^3), temperature of maximum density (11.6 °C) and heat of vaporization (10,864 cal/mol). It has been accepted that two independent physiological effects occur when a living system is exposed to D_2O: i) an isotope exchange effect on functional proteins and ii) a solvent isotope effect on ionic conductivity, meaning that mobility of monovalent cation is reduced to <20% (Bass & Moore, 1973). When H is replaced with D in the biological molecule, the C-D bond is about 10 times stronger than the C-H bond, which means the C-D bond is more resistant than the C-H bond. O-D, N-D and S-D bonds are stronger than the corresponding protonated forms (Katz, 1965; Thomas, 1971). This H/D exchange effect on the functional protein can be observed by using thermal imaging and calcium imaging (Hirakura et al., 2011). In the following sections, we will discuss these two effects from the physical aspect and will propose a thermo-dynamical model for elucidation of water-protein interaction.

2. Effect of D$_2$O on the ion channel activity

According to the list of ionic conductance appearing in a previous report (Bass & Moore, 1973), the mobility of proton in H_2O is 1.44 times higher than that in D_2O (H/D ratio). The H/D ratio of other cations, K^+ and Na^+, was also higher in mobility as 1.35 and 1.47,

respectively. Interestingly, the H/D ratio in proton mobility in ice is enormously high (= 6.25). Therefore, it will be important to study the inside structure of the channel pore by measuring the proton current under D_2O medium.

2.1 Proton channel

The latest data on D_2O effect on proton conductance was obtained by DeCoursey and Cherny (DeCoursey & Cherny, 1997), which showed the H/D ratio in proton conductance to be estimated at 1.9. Simultaneously they obtained some other results, such as (a) D^+ permeated proton channels, (b) the relative permeability of proton channel, however, was 10 times more greater for D^+, (c) D^+ regulated the voltage dependence of the proton channel gating like H^+, (d) D^+ current induced with depolarization was 3 times slower than that for H^+ current, but deactivation was at most 1.5 times slower in D_2O. Both activation and inactivation for D^+ current were found to be slower in general. Does proton go through water in the channel pore or on the surface of channel protein? In order to answer to this question we need to consider how proton flows in the water. We need to consider that proton flow in the hydrated protein appears abruptly when water content exceeds critical concentration, which was predicted by percolation theory (Careri et al., 1990).

Namely, proton is flowing through the hydrated water layer on the protein surface. Since the critical value of water content for proton flow is independent on the pH values, a model for proton flow will be clear by the measurement of pH dependency. Unexpectedly, DeCoursey and Cherny (DeCoursey & Cherny, 1997) found the threshold of applied voltage to generate proton current was pH dependent, while proton conductance was pH independent. What is the role of the proton channel in the cell? We will discuss this problem in the next section.

2.2 Na^+ channel

As was described previously, the H/D ratio in conductance of Na^+ is 1.35 (Schauf & Bullock, 1982). Different from other ionic currents, when the Na^+ channel is abruptly depolarized, a small outward current precedes the Na^+ current. This small displacement current is asymmetric, named "gating current", because it is always associated with the opening of Na^+ channels. The gating current can be isolated by blocking Na^+ current by tetrodotoxin (Armstrong & Bezanilla, 1973). About 30 years later, the gating current was found to be decreased by about 30% when H_2O was changed to D_2O (Landowne, 2000). Na^+ current itself decreased as observed previously by other researchers (Meves, 1974), although these two types of current could be separated by a drug. The interpretation of the gating current by Landowne is that D_2O slowed the rate of the conformational change of channel protein by 30%, thus reducing the amplitude of the gating current and increasing the time required to open the channel due to the high viscosity of D_2O near the channel protein (Landowne, 2000). K^+ channel conductance was also studied by Schauf and Bullock, and H/D ratio in K^+ conductance was reported as 1.47, which was almost the same as that of Na^+ (1.35) (Schauf & Bullock, 1980). Accordingly, the D_2O exchange effect for both channels may be the same as each other. In order to examine the D_2O effect on Na^+ and K^+, high-K^+-induced depolarization of membrane potential was measured using AtT-20 cells (Ikeda et al., 2004). As shown in Fig 1, when the cell is exposed to 30mM KCl, the membrane potential was depolarized from –69mV to –32mV (Fig. 1A). D_2O treatment shifted resting potential slightly

(~ +4.4mV, about 10% of 30mM K+-induced depolarization). This difference agreed well with the channel conductance difference between Na+ and K+ as reported by Shauf and Bullock (~9%) (Schauf & Bullock, 1980).
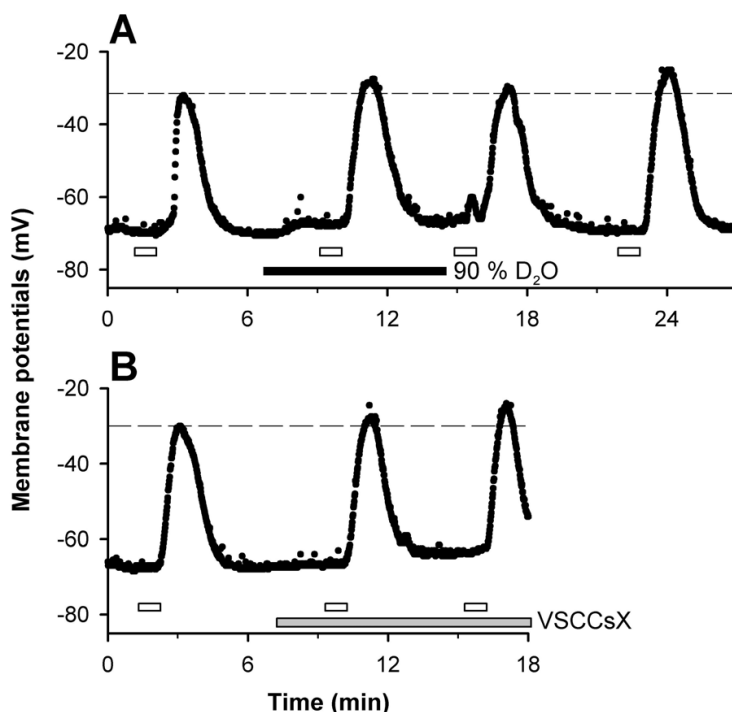

Fig. 1. Effects of 90% D_2O solution on high-K+-induced membrane depolarization. Open bars = 30mM K+, black bars = 90% D_2O treatment, grey bar = a mixture of voltage-sensitive Ca^{2+} channel blockers (VSCCsX). (Ikeda et al., 2004)

2.3 Ca^{2+} channel

An initial study on the D_2O exchange effect of the Ca^{2+} channel protein was carried out by Andjus et al. using inter nodal cells of the fresh water alga, which was known as a unique system before the discovery of the patch clump method (Andjus et al., 1994). They reported that there was no D_2O exchange effect on channel gating in the change of the resting potentials made by different concentration of KCl solutions. The single channel conductance was also unchanged, but open channel probability at 10mM KCl was increased irreversibly. The asymmetric distribution of D_2O (extracellular) and H_2O (intracellular) across plasma membrane was able to activate the Ca^{2+} channels of these cells. They suggested that transient osmotic-like stress produced by the rapid trans-membrane diffusion of D_2O may mediate the Ca^{2+} channel activations (Brooks, 1937).

We estimated the kinetics of the voltage-sensitive Ca^{2+} channel by whole cell patch using AtT-20 (Murine anterior pituitary corticotroph tumour) cells. The peak Ca^{2+} current recorded in H_2O-solution-filled electrode was -61.3pA, while the current recorded in D_2O-filled electrode at the same holding potential was significantly reduced to –15.4pA. Here, the H/D ratio of Ca^{2+} conductance was 3.98, which is 2.7 times higher than that of K+ conductance (1.47) and 2.9 times higher than that of Na+ conductance (1.35). Since Ca^{2+} is a divalent cation, the effective values will be halved (1.35 times higher than H/D ratio of K+

conductance and 1.45 times higher than that of Na^+ conductance). Significant difference in H/D ratio of the conductance between Ca^{2+} and Na^+ (or K^+) cannot be explained by a simple model. To elucidate this unique feature of the Ca^{2+} ion in the living cell, we attempted further experiments. The results obtained are shown in Fig. 2.
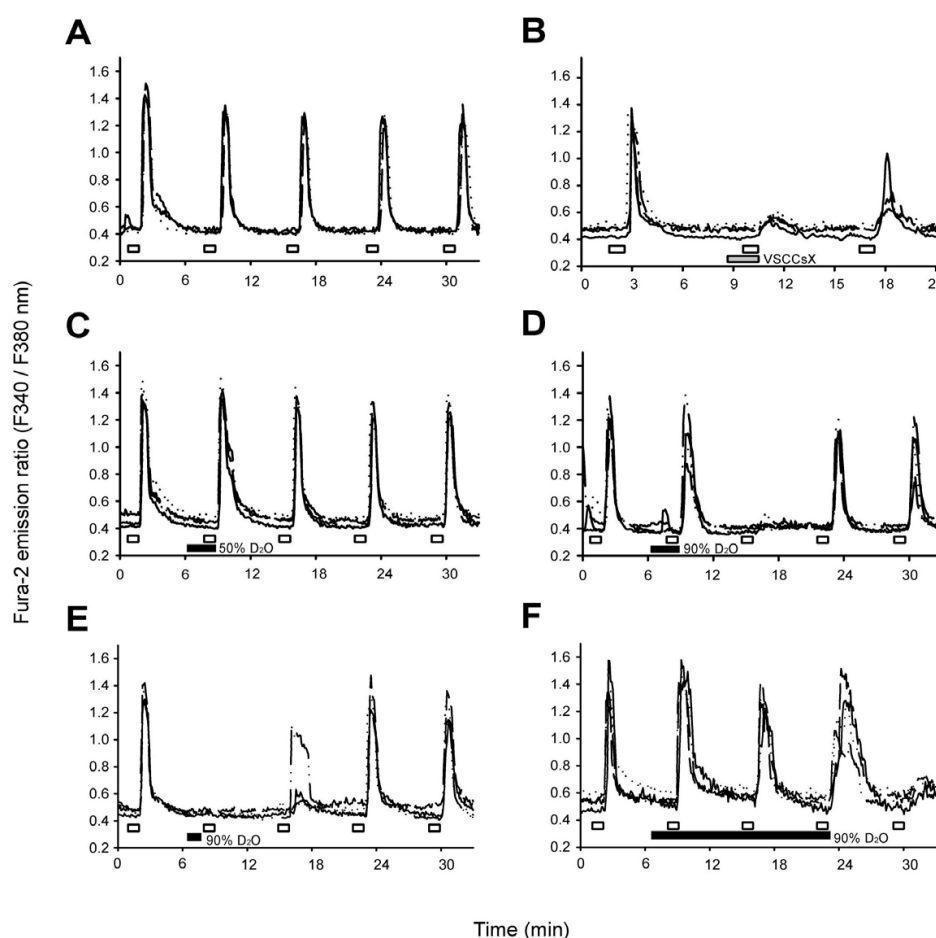

Fig. 2. The effects of D_2O on high-K^+-induced Ca^{2+} influx in AtT-20 cells. Open bars = 30mM K^+, grey bar = voltage-sensitive Ca^{2+} channel blockers (VSCCsX), black bars = 90% D_2O medium. (Ikeda et al., 2004)

As shown in Fig. 2, high-K^+-induced depolarization made a reproducible and rapid increase in the intracellular Ca^{2+} concentration (Fig. 2A). This Ca^{2+} elevation is primarily due to the Ca^{2+} influx through voltage-sensitive Ca^{2+} channels (VSCCs), because typical Ca^{2+} channel blockers, such as nifedipine (10µM) and ω-conotoxin (100nM), significantly reduced (~90%) the amplitude of the high-K^+-induced Ca^{2+} elevation (Fig. 2B). High-K^+-induced Ca^{2+} influx during treatment with D_2O-contained extracellular solution was not changed, whereas Ca^{2+} responses after wash out of D_2O was found to be reduced significantly. But this reduction was recovered gradually within several minutes after D_2O wash out (Fig. 2D). A 1 minute treatment with D_2O solution was sufficient to inhibit such a high-K^+-induced Ca^{2+} entry (~90% reduction; Fig. 2E), whereas longer D_2O treatment (up to 17 min), unexpectedly, showed a slight inhibitory effect (~8% reduction). Based on the data shown in Fig. 1, Fig. 2 and the voltage clamp experiment, it was concluded that the differences between a strong

inhibition of Ca^{2+} channel by transient D_2O treatment and a slight inhibition with longer D_2O treatment will be explained by asymmetric distribution of D_2O (inside) and H_2O (outside) in a transient and stable D_2O effect, respectively. Consequently, the H/D ratio on the Ca^{2+} channel is estimated as ~1.08 from Ca^{2+} imaging data and ~1.20 from electrophysiological data.

How can the asymmetric distribution of D_2O and H_2O modulate the Ca^{2+} channel? The involvement of stress-sensitive mechanisms can be excluded, because cell shape change was not observed. The solvent isotope effect was also unlikely to explain the delayed effect of D_2O. Tentatively, it is likely that local unbalanced distribution of D and H in the O-H bonding of the channel protein is the cause. This D/H competition was already observed by Vasdev et al. (1994) with the anti-hypertensive effect of D_2O related to L-type Ca^{2+} channel conductance in myocyte. Proudhon et al. showed that D^+ could compete with H^+ for a single site in the L-type Ca^{2+} channels of guinea pig ventricular myocytes (Proudhon et al., 1994). It was thought that binding and unbinding of protons to this site were essential for Ca^{2+} movement (Kushner et al., 1998). This model can be expanded to the case of Na^+ channels as well as K^+ channels. The mechanism of this phenomenon will be discussed further in section 5.

3. Effect of D_2O on the cytoskeleton

As a solvent, D_2O increases the stability of proteins and other molecules, such as heliozoan microtubule formation, by hydrophobic bond formation (Marsland et al., 1971) and it has been used as a polymerizer of tubulin in a number of systems.

3.1 Tubulin is stabilized by D_2O

A number of studies on the effect of D_2O on protein aggregation have been conducted since the 1970s. It was almost accepted that D_2O stabilized the aggregated form of oligomeric proteins (Baghurst et al., 1972; Bonnete & Zaccai, 1994; Henderson et al., 1970). In 1999, Chakrabarti et al. reported clear results indicating that unstable tubulin protein was stabilized in D_2O and they proposed a mechanism whereby D_2O can have an effect on the conformational step or steps of hydrophobic force disruptions (Chakrabarti et al., 1999). Furthermore, they observed that D_2O stimulated formation of microtubule from tubulin as was observed previously (Ito & Sato, 1984). More interesting, when 8% of DMSO is added in the D_2O solution, tubulin polymerized as a ribbon structure rather than microtubules.

Although classical interpretations of D_2O-induced inhibition of cellular secretion have been based on D_2O-mediated stabilization of microtubules (Hill & Rhoten, 1983; Malaisse-Lagae et al., 1971; Montag & Umanskii, 1976), the effect of D_2O on cytoskeleton appear to be more prevalent and varied. In order to study relationships between cellular function and microtubule aggregation by D_2O, several researchers investigated the effect of some agents on the interaction between D_2O and tubulin. Urata et al. studied the effect of demecolcine (microtubule depolymerizing agent), taxol (microtubule stabilizing agent) and cytochalasin B&D (microfilament blocker) on the IgE-mediated Ca^{2+} influx, arachidonic acid and histamine release in rat basophilic leukaemia cells (Urata et al., 1989). They concluded that microtubule aggregation may be related to the process of secretion. Also, D_2O enhanced secretion of histamine from cultured mouse spleen cells and other mediators from homologous mast cells (Sulowska & Wyczolkowska, 1991). By exposing covalent oligomers

of IgE to RBL-2H3 cells (a rat basophilic leukaemia tumour cell), substantial increases in a secretion of histamine was demonstrated by using D_2O in the medium (Maeyama et al., 1986). The differential effects of microtubule-altering agents (vinblastine; VB) on beta-cells during development were shown by comparison with D_2O and they speculated that microtubule is not coupled physico-chemically to other molecules in insulin secretion at day 17 of gestation during development (Hill & Rhoten, 1983). All these data suggest that tubulin microtubule transition is highly regulated by exchange of H/D. That means that the transition may be related to the mass difference between H (= 1.007) and D (= 2.014). The effect of mass difference of water molecules on the cell function will be discussed in section 5.

3.2 Effect of D_2O on the actin structure

Zimmermann et al. reported that D_2O induces the redistribution of filamentous actin (F-actin) and changes the morphology of human neutrophil granulocytes (Zimmermann et al., 1988). More recently, Omori et al. assessed the effect of D_2O on microfilaments and on in vivo actin polymerization using BALB/3T3 cell (Omori et al., 1997). They observed that the cells' stress fibres in the peripheral region became thick and distinct from other regions after being exposed to D_2O (>30%), while the quantity of perinuclear microfilaments was drastically reduced. Cytoplasmic F-actin was found to be increased with the stress fibres. Cell locomotion activity was suppressed in a D_2O concentration. The rate of actin polymerization was accelerated when purified globular actin (G-actin) was polymerized in D_2O. They concluded that alteration of stress fibres in cultured cells may be caused by a direct effect of D_2O on cellular microfilament dynamics (Omori et al., 1997). One possible mechanism underling D_2O-induced actin filament redistribution may involve H to D exchange in globular protein (Hermans & Scheraga, 1959; Scheraga, 1960), which results in a more stable protein structure (Karasz & Gajnos, 1976; Sing & Wood, 1976).

We tested this assumption by Ca^{2+} imaging method with the use of AtT-20 cells. Initially, we examined rhodamine-phalloidin labelling of actin filaments and immunostaining of β-tubulin.

As shown in Fig. 3, actin filaments were observed as filamentous structures that were found to be more highly concentrated in the cell processes than in cell soma (Fig. 3A & 3Ba). Treatment with D_2O-containing solution for 5 minutes immediately increased the amount of actin filaments in the cell soma and eliminated its filamentous structure (Fig. 3A). The relative amount of actin filaments in the cell process was decreased with D_2O treatment (Fig. 3Bb). Longer (15 minutes) treatment with 90% D_2O resulted in an increase in the actin filaments in the cell processes again, however, the filamentous structure was still lacking (Fig. 3A). After D_2O wash out, the filamentous structure of the actin filament recovered, but the shape of the filaments in the cell processes was similar to that of varicose filaments (Fig. 3A & 3Bc). The original shape of the actin filaments did not recover until 30 minutes after the D_2O wash out. Despite the marked changes in the distribution and structure of actin filament, those of β-tubulin (Fig. 3A) or those of neurofilament-M were not changed by D_2O treatment. The results described above indicate that among several cytoskeletal components, D_2O affected actin filaments especially. The effect of D_2O on the amount of actin filament was found to be transient (15 minutes), which is consistent with the results of the previous report (Omori et al., 1974).
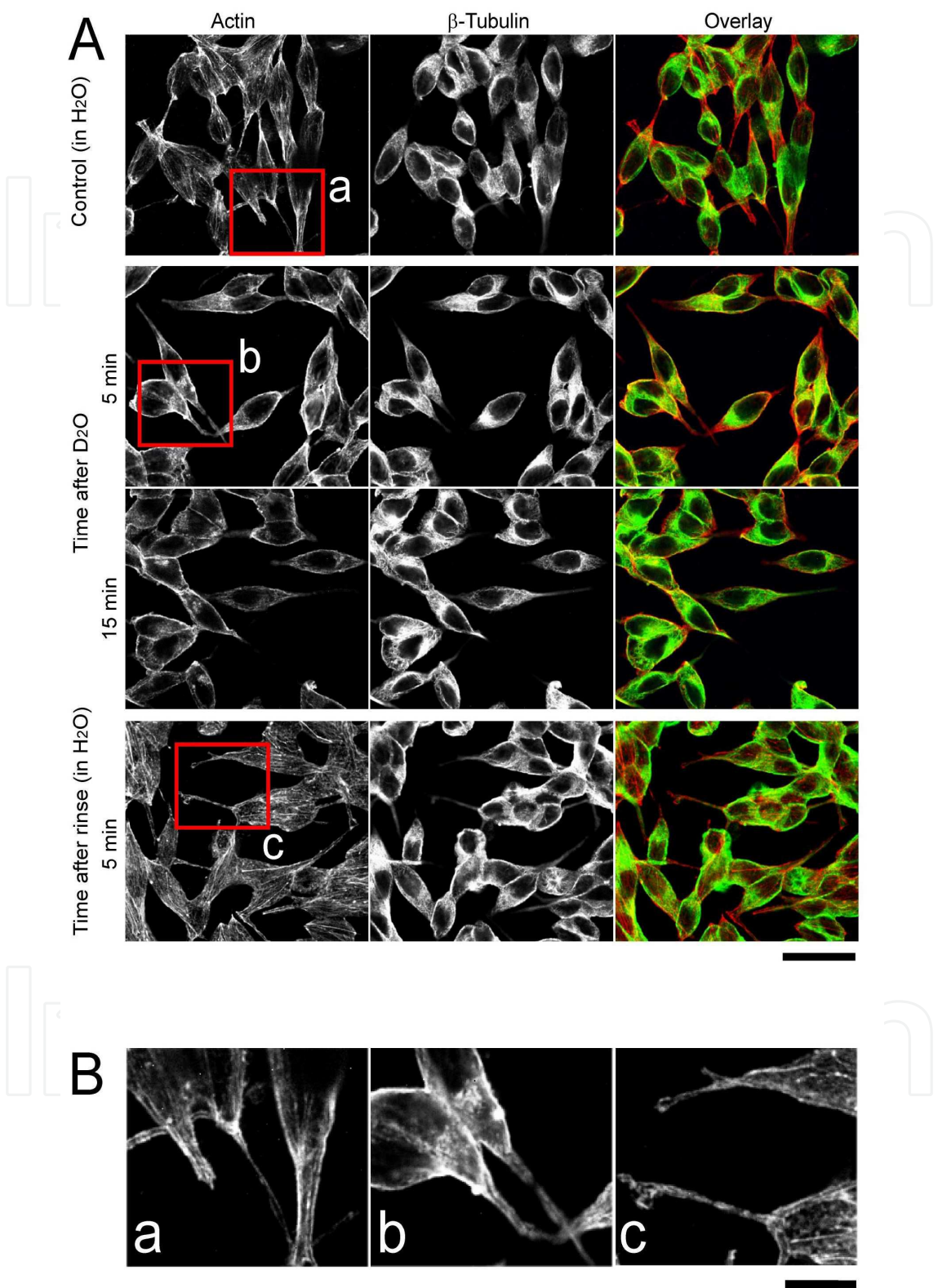
Fig. 3. Effects of 90% D₂O treatment on actin filaments (rhodamine-phalloidin labelling, red) and β-tubulin (FITC-labelling, green) in AtT-20 pituitary cells. The typical changes in the distribution of actin filaments (marked as red frame a-c) are enlarged in (B). Bar = 50μm in (A), 20μm in (B). (Ikeda et al., 2004)

3.3 Novel hypothesis for the molecular mechanism of interaction between channel protein and D_2O

More than 40 years has passed since the beginning of D_2O study, but the molecular mechanism of the interaction between D_2O and cytoskeleton remains unresolved. Therefore, we would like to attempt making a novel model based on the water structure near the cytosol protein proposed by Mentre and Hui Bon Hoa (Mentre & Hui Bon Hoa, 2001). First of all, we have to recall that the H/D ratio of the proton mobility in water is 1.44, while that in ice is 6.25 (4.3 times higher in the ice!). This is the most important numerical value to explain the difference of cellular function between H_2O and D_2O, because, in general, water structure on the surface of protein and lipid membrane is known as bound water, which has a similar structure to ice. According to Kellenberger, water structure on the surface of high polymer is quite different from free water (Kellenberger, 1991). It is called structural water, polarized water, strained water or vicinal water, which form the hydration shell of the surface of high polymer. Water molecules will be structural by hydration with a dissociated group and hydrogen bonding with a polarized group. Attention must be paid so that the structural water can convert chemical energy to mechanical energy, transfer several types of signals and make a biological structure. The structure and characteristics of bound water is very similar to those of ice. It is difficult to be frozen and sublimated. Furthermore, solubility of bound water is changed by hydrated ion, such as Na^+, K^+ and Ca^{2+}, due to exclusion. The order of exclusion follows the Hofmeister series. For example, the exclusion values of Na^+ and Ca^{2+} can be excluded more than that of K^+, which will induce a higher H/D ratio for Ca^{2+} to some extent.

3.4 New hypothesis for interaction between D_2O and cytoskeleton

Albrecht et al. proposed that linear structures in the cell, such as cytoskeleton, transmitted and propagated a signal by changing their structure (Albrecht et al., 1990). Dimer of tubulin α and β can be exchanged in a seesaw action and can make different structures from $\alpha\beta$ to $\beta\alpha$ by the signal initiation, called flipping. This flipping model is available for the explanation of structural change associated with H/D changes. The signal transfer along actin fibre is more complex because of actin binding to trans-membranous fibronectin. When the cell is moving, such as microglia, actin is supported by fibronectin as a fulcrum. Another example is skeletal muscle, where several proteins, such as myosin, dynein and kinesin, can move with the structural change of water induced by Ca^{2+} concentration change near the actin fibre. Thus, cellular signalling will be transferred along actin fibre or microtubule with the assistance of water structure change associated with Ca^{2+} concentration change.

4. Molecular mechanism of hysteresis in the D/H and H/D exchanges in the protein structure

During D/H and H/D exchange experiment, we have noticed that the H/D exchange rate is faster than that of D/H in any type of experiment. In order to confirm the anisotropic effect, a series of new methods was carried out using metabotropic glutamate receptor type 1 (mGluR1)-expressing CHO cells by Ca^{2+} imaging (Hirakura et al., 2011). The first hysteresis experiment was to measure the exchange rate of H_2O to D_2O by utilizing differences in fluorescence intensity of fura-2 in H_2O and D_2O under the same concentration of Ca^{2+}.
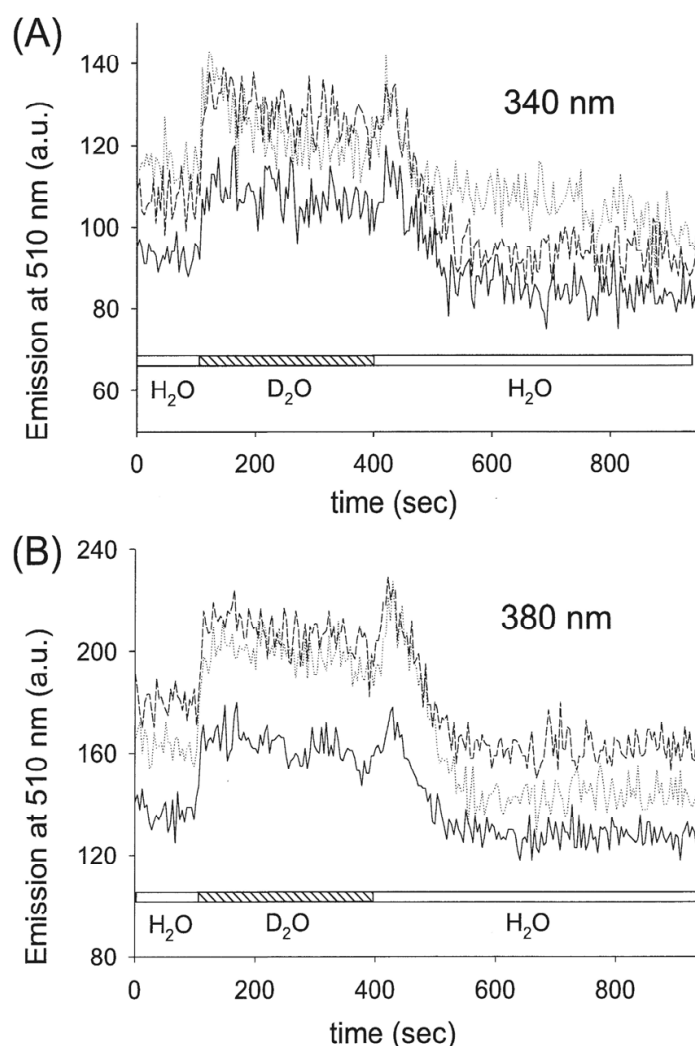
Fig. 4. Measurement of flow rate of D_2O entering the cell and the D/H exchange rate for fura-2 molecules in Ca^{2+}-free media. (A) Ex =340 nm, (B) Ex = 380 nm. (Hirakura et al., 2011)

As shown in Fig. 4, when the external solution was changed from H_2O to D_2O medium, the fluorescence intensity of fura-2 was increased at 380nm excitation. In contrast, when the external solution was changed from D_2O to H_2O, a slight rebound signal appeared and then the intensity declined slowly. The time of fluorescence increase in H to D exchange is about 5 sec, while the decaying time is more than 100 sec. Another example of hysteresis was observed more clearly as shown in Fig. 5.

In these experiments, exchange of extracellular medium was carried out with changes from H_2O to D_2O and back to H_2O at 5 minute intervals and the cells were stimulated with dihydroxyphenylglycine (DHPG; agonist of mGluR1) to induce Ca^{2+} elevation. DHPG-induced Ca^{2+} responses were relatively constant in D_2O, while Ca^{2+} responses were completely blocked during 5 minutes of incubation in H_2O after an incubation in D_2O medium (Fig. 5A). With a 15 minute incubation in H_2O medium after 5 minutes of exposure to D_2O, DHPG-induced Ca^{2+} responses partially recovered (Fig. 5B). When the incubation time in H_2O medium was increased to 30 minutes, Ca^{2+} responses were fully recovered (Fig. 5C & 5D).
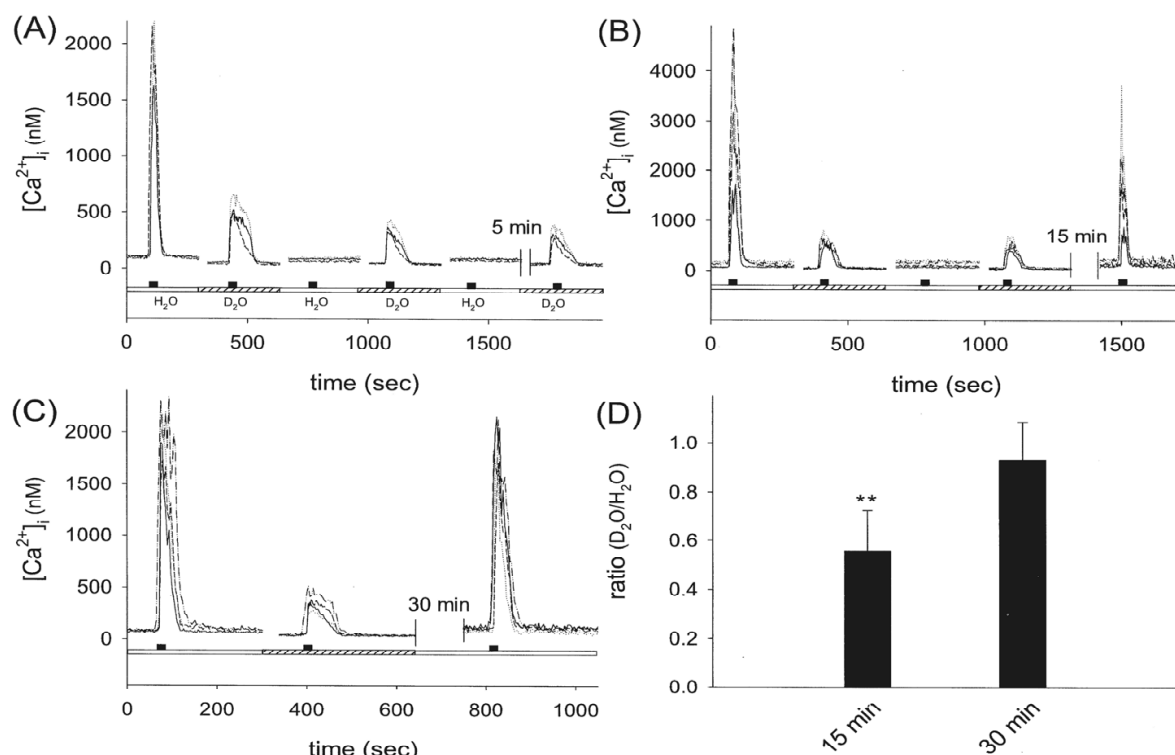
Fig. 5. Hysteresis of DHPG-induced Ca^{2+} responses induced by D/H exchange. Open bars = H_2O, shaded bars = 90% D_2O medium, small black squares = DHPG (30μM). Summary of the Ca^{2+} responses are shown in (D). **p < 0.01 (Hirakura et al., 2011)

It is reasonable to explain that these data demonstrate the incubation period in H_2O medium is a critical factor for the recovery after D_2O exposure. When the cells were incubated in D_2O without agonist stimulation, however, DHPG-induced Ca^{2+} responses seemed normal. These results suggest that receptor stimulation is associated with large structural change of receptor that promotes deuteration of the intracellular portion in the receptor protein, which is consistent with a previous report showing that D/H exchange was facilitated during photo-activation of rhodopsin (Rath et al., 1998). The backbone structure of rhodopsin, G-protein-coupled photo-receptor with seven membrane spanning regions, becomes more accessible to D_2O during photo-activation. After stimulation, the receptor protein is more stable and resistant to D/H exchange. The increased stability of the deuterated protein may contribute to the prolonged stable state of the receptor. This hypothesis can be expanded to various types of receptor dynamics, including mGluR1 in the CHO cells. A similar hysteresis effect of H/D exchanges on the voltage-dependent Ca^{2+} channels was also reported previously (Ikeda et al., 2004). Thus we suggest that such hysteresis effect may be due to impairment of D/H exchanges. This may be a typical isotope exchange effect, which is due to a difference in the stability of proteins: deuterated proteins are more stable than protonated protein (Chakrabarti et al., 1999; Omori et al., 1997). Among the many known effects due to the difference in reactivity of D^+ and H^+, the most relevant to the effect observed in this study may be the zero-point energy differences. A quantum chemical calculation indicates that the isotopic substitution of hydrogen by deuterium lowers total energy of hydrophilic amino acids, because of the decrease in zero-point vibration energy
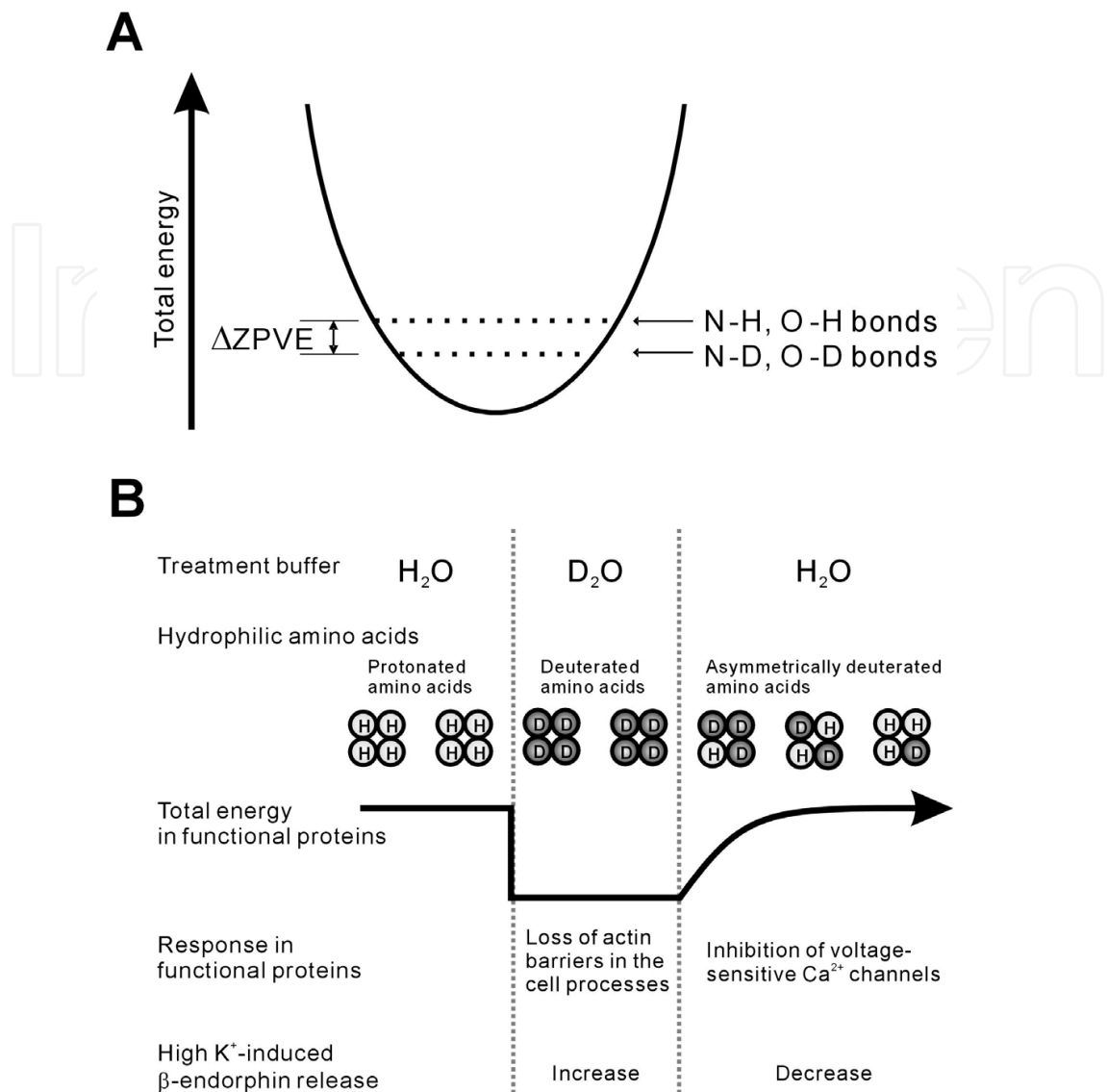
A

B

Fig. 6. Schematic illustration of changes in the zero-point vibrational energy and the energy differences between protonated and deuterated amino acids when hydrogen is exchanged by deuterium in hydrophilic amino acid residues of proteins. (A) Solid curve denotes the electronic energy of the system. Single deuterium substitution for hydrogen reduces approximately 2.1 kcal/mol (ΔZPVE) in hydrophilic amino acids. Total vibrational energy reduction in functional proteins depends on the number of deuterated amino acids replaced. (B) For the energy differences between protonated and deuterated amino acids, replacement of H_2O buffer to D_2O buffer immediately exchanges protonated amino acids to deuterated amino acids while replacement of D_2O buffer to H_2O buffer slowly exchanges deuterated amino acids and thus produces long-lasting transient states with asymmetrically deuterated amino acids. The increase in the high K^+-induced β-endorphin release is in the phase of deuterated amino acids and the most probable cause is due to the loss of actin filament barriers in the cell processes, although this effect coincides with many other effects including solvent isotope effects. On the other hand, the decrease in the high K^+-induced β-endorphin release is in the phase of asymmetrically deuterated amino acids and the most probable cause is the complete inhibition of voltage-sensitive Ca^{2+} channels. (Ikeda et al., 2004)

(Ikeda et al., 2004). Therefore, it is likely that cells rapidly reach equilibrium after an exchange of H_2O to D_2O, but take a longer time to reach equilibrium after switching from D_2O back to H_2O. Within the hydrophilic amino acids, a relatively large zero-point energy was found in tyrosine that is known as a critical residue for the voltage sensor of L-type Ca^{2+} channels (Bodi et al., 1997). Thus, this may produce a relatively long lasting transient state in voltage-sensitive Ca^{2+} channels with asymmetric deuterated amino acids after treatment by D_2O medium that will inhibit the Ca^{2+} channel activity (Fig. 6).

5. Role of proton in the regulation of functional proteins

As described above, the H/D exchange experiment was found to be effective to elucidate the role of water in the functional protein. When H is replaced by D, the structure and function of protein was largely changed, although chemical characteristics of D_2O are the same as H_2O. These facts facilitate us to consider carefully the physical properties of H_2O and the physical interaction between H_2O and protein. Here, we will discuss the role of proton in the ion channel and cytoskeleton in the cells, and hysteresis in H/D exchange; it was tentatively proposed that the channel conductivity was regulated by the local unbalanced distribution of D^+ and H^+ in the O-H residue of the channel proteins. Stabilization of cytoskeleton in D_2O will be due to the contribution of structural water and significance of the Hofmeister series. On the hysteresis effect observed in H/D exchange experiment, the significance of zero-point energy was proposed. These three models seem different to each other, but there is a possibility of making a simple model if we accept structural water layer model on the protein surface. Prior to making a novel model, drastic concept for cellular signalling is needed as proposed by Tsong (Tsong, 1989).

5.1 Electro conformational coupling (ECC) model for cellular signalling and energy transduction

In 1989, Tsong insisted that cells can communicate with each other by dispatching and receiving electro-magnetic signals. He observed that Na^+/K^+ ATPase (transmembrane ion transport protein) is sensitive to electric signal and it is most sensitive for alternative electric field of 1000 Hz 20V/cm. Based on these facts, he proposed his theory to explain communication between protein molecules in the cell; all kinds of proteins can respond to electric, electro-magnetic (EM) and acoustic oscillation. This theory was supported by many experimental results. Pulsed EM field was found to promote DNA, RNA and protein biosynthesis (Becker, 1981; Goodman et al., 1983; Pilla et al., 1987). The activity of many membrane enzymes are affected by weak EM signals (Adey, 1986; Blank, 1987). This model postulated that a protein can undergo conformational changes by a coulombic interaction with an oscillating electric field. When the frequency of the electric field matches the kinetic characteristics of the conformational transformation reaction, a phenomenological oscillation among different conformers of the enzyme is induced. He also insisted that this ECC model was consistent with the electric property of the most membrane proteins (Tsong & Astumian, 1987). This model is very attractive for the explanation of the cell-to-cell interaction and intracellular signalling by protein networks. However, some points of modification are needed, because this hypothesis cannot explain completely the functional change by H/D exchange. Therefore, we will try to make a novel model including H/D exchanges.

5.2 Proton signalling model for cellular function

The ECC model described above seems to assume electron transfer occurs in the protein molecule. But it is already accepted by many researchers that the transfer of electron along protein is accomplished by a series of redox centres incorporated into the protein structure or along a chain of conjugated orbital (Tuszynski, 2003). This protein-electron carrier system consists of a protein localized within a lipid bilayer having a single redox centre. The redox centres are usually prosthetic-group-containing non-protein molecules that have conjugated orbital systems and often incorporate metal ion. Furthermore, it has been proposed that protein is insulator at physiological temperature and that electron transport is mediated by proton. In this model, an electron is moving along the protein backbone by protons. Therefore, it is reasonable to assume that such a conformational dynamics of protein is depending on the solvent in *in vitro* experiment. Such a protein–solvent structure should be treated as a single system whose behaviour can be controlled by the environment (Tuszynski, 2003). If that is the case, proton signalling by proton transport will be likely. Accordingly, it is worth noting that structural water in the vicinity of protein molecule has a structure similar to ice. First of all, attention should be given to the fact that ice and water have very low conductivity for electron, while it is a good conductor for proton. In 1962, Grottus proposed an initial model for proton transfer in the water and the model was presented by Klotz in his book (Klotz, 1962). When one signalling proton reaches to the tip of the chain of water molecules, the proton is taken by the first water molecule. The first water molecule transfers one proton of two protons to the second one. Thus, the same type of proton transfer is repeated until the end of a line of water molecules, and then the proton will be released. In this process, the proton itself is not transferred from first water molecule to the last one, but chain-like molecular structure is changed successively. This is a typical type of salutatory conduction of proton in the water (or ice). Since the thickness of structural water layer is very thin (0.2 ~ 1.0 nm), the proton signalling in the water layer was a hypothesis in the 1980s, but it is measureable at present. Let us discuss the water layer inside of the channel pore and surface of cytoskeleton more deeply.

5.2.1 How ion channel permeate ion

According to a typical text book published in 2008, conductivity of the ion channel is formulated based on the diffusion theory (Dale Purves et al., 2008). In famous experiments performed by Hodgkin and Katz using squid giant axon, they found that the value of resting potential became less negative as external K^+ concentration was raised. When the external K^+ concentration was raised high enough to equal the concentration of K^+ inside the neuron, thus K^+ equilibrium potential was 0 mV, the resting membrane potential was also 0mV. In short, the resting membrane potential was not varied exactly 58 mV by ten-fold change in K^+ concentration. They thought the deviation from theoretical value may be due to the contribution of another ion, such as Cl^- and Na^+. But there is clear evidence that the inside of a K^+ channel is covered by a structural water layer and the iceberg structure of water can be disrupted by K^+. Therefore, the resting membrane potential of nerve cell membrane is approaching to Nernst equation because the iceberg structure of the water layer becomes free water at higher concentrations of K^+. If it is the case, ion selectivity of ion channel does not depend on the size of hydrated ion, but on that of metallic ion.

5.2.2 Relationship between shape change of cytoskeleton and iceberg water.

An explanation of the complex behaviour of cytoskeleton is still very difficult. The long fibres of the cytoskeleton are polymers composed of protein subunits. In this article, we will focus on tubulin (microtubules) and actin (microfilament). The tubulin that polymerizes to form microtubule has two isoforms (α-tubulin and β-tubulin) and forms a hetero dimer. The α- and β-tubulin monomers each consist of two β-sheets flanked by α helices to each other. The elementary building block of a microtubule is α and β hetero dimer whose dimensions are 4 by 5 by 8 nm that assemble into a cylindrical structure typically of 13 protofilaments. The outer diameter of a microtubule is 25 nm and the inner diameter is 15 nm. Microtubules are larger and more rigid than actin microfilaments and intermediate filament, and thus serve as major architectural struts of the cytoplasm. Above a certain tubulin concentration threshold, microtubule ensembles show a quasi-periodic regular pattern of damped oscillations (Carlier & Sellier, 1987). It is intriguing to know how the stochastic individual behaviour may change into smooth collective oscillations at high tubulin concentration (Mandelkow & Mandelkow, 1992).

Actin is one of the most abundant proteins in eukaryotic cells. G-actin and F-actin are reversible structures of actin. F-actin is a polymer of G-actin. Actin can be found in non-muscle cells and often is associated with other proteins in the cytoskeletons, where actin works as a dynamic component. G-actin monomers assemble into F-actin filaments in two-stranded geometry. The polymerization of F-actin from G-actin is a largely monotonic process dependent on the concentration of ATP. Once G-actin is assembled, microfilaments have diameters of about 8 nm. Microfilaments are often found with the lattice configuration near the leading edges of growing or motile cells, where they provide greater stability to the newly formed regions. New actin filaments are nucleated at the leading edge of cell growth and trailing microfilaments are disassembled.

These dynamic properties of cytoskeleton can also be explained by structural water theory. The concept of cluster of water molecules proposed by Watterson [Watterson, 1991] is predominant (Watterson, 1991). Water molecules have a resonance due to co-laborational work by hydrogen molecules, and then strong oscillation will be generated. This resonance will not be expanded to the large scale because of disturbance by thermal agitation, but it may be a cause of surface tension of the water molecules and the ordered structure of water, which is called "cluster". The cluster is thought to have the oscillations, whose wave length is calculated as 3.4 nm (~with network [addition of ion]), this impact will be spread out in the network rapidly just like a propagated wave of sound. The wave will decay rapidly in the region with high viscosity and it propagated far away. Therefore, the wave propagation mode is disturbed largely when ion is added to the cluster. Consequently, dynamic shape change can be induced by abrupt application of ion or other molecules.

6. Concluding remarks

In this article we demonstrated the change of activity of ion channels and glutamate receptor, and the structural change of cytoskeleton by H/D changes. These cellular changes were always associated with hysteresis effect of the change of incubation medium from H_2O to D_2O and vice versa. Initially, we tried to approach these phenomena independently, such as zero-point energy difference between protonated and deuterated protein. Finally, we

reached the reasonable conclusion that the difference between H_2O and D_2O might be caused by a structural water or proton. If we introduce proton signalling pathway in the structural water in the vicinity of protein, all types of changes in the protein function could be explained without contradiction.

It is worthwhile to note that this model can be interpreted as a docking of the water layer model (Mentrè, 1995) and ECC model (Tsong, 1989), and improved them to some extent to explain reasonably the imaging data recently obtained (Hirakura et al., 2011; Ikeda et al., 2004).

7. Acknowledgement

8. References

Adey, W. R. (1986). The sequence and energetics of cell membrane transducing coupling to intracellular enzyme systems. *Bioelectrochemistry and Bioenergetics*, vol.15, pp447-456.

Albrecht, T., Boldogh, I., Fons M., AbuBakar S., & Deng C. Z. (1990). Cell activation signals and the pathogenesis of human cytomegalovirus. *Intervirology*, vol.31(2-4), pp68-75

Andjus, P.R., Kataev, A.A., Alexandrov, A.A., Vucelic, D., & Berestovsky, G.N. (1994). D_2O-induced ion channel activation in Characeae at low ionic strength, *The Journal of membrane biology*, vol.142, No.1, pp43-53

Armstrong, C. M. & Bezanilla F. (1973). Currents related to movement of the gating particles of the sodium channels, *Nature*, vol.242, No.5398, pp459-461

Baghurst, P.A., Nichol, L.W. & Sawyer, W.H. (1972). The effect of D 2 O on the association of -lactoglobulin A. *The Journal of Biological Chemistry*, Vol.247, No.10, pp3199-3204

Bass, L. & Moore, W.J. (1973). A simplified cooperative model of excitable membranes. *The Journal of Membrane Biology*, vol.12, N0o.4, pp361-366

Becker, R. O. ed (1981). *Mechanisms of Growth Control*, Charles C Thomas Pub Ltd, Springfield .

Blank, M. a. F., E. (eds) (1987). *Mechanistic Approaches to Interactions of Electric and Electromagnetic Fields with Living Systems* Plenum Press.

Bodi, I., Yamaguchi, H., Hara, M., He, M., Schwartz, A. & Varadi, G. (1997). Molecular studies on the voltage dependence of dihydropyridine action on L-type Ca2+ channels. Critical involvement of tyrosine residues in motif IIIS6 and IVS6. *The Journal of Biological Chemistry*, vol.272, No.40, pp24952-24960

Bonnete, F. & Zaccai, G. (1994). Small angle neutron scattering, total cross-sections and mass density measurements of concentrated NaCl and KCl solutions in H2O or D2O. *Biophysical Chemistry*, vol.53, No. 1-2, pp69-75

Brooks, B. T. (1937). Robert Kennedy Duncan, *Science*, vol.85, No.2212, pp489-491.

Careri, G., Consolini, G. & Bruni, F. (1990). Proton tunneling in hydrated biological tissues near 200 K. *Biophysical Chemistry*, vol.37, No. 1-3, pp165-170

Carlier, J.P. & Sellier, N. (1987). Identification by gas chromatography-mass spectrometry of short-chain hydroxy acids produced by Fusobacterium species and Clostridium innocuum. *Journal of Chromatography*, vol.420,No.1, pp121-128

Chakrabarti, G., Kim, S., Gupta, M.L., Jr., Barton, J.S. & Himes, R.H. (1999). Stabilization of tubulin by deuterium oxide. *Biochemistry*, vol.38, No.10, pp3067-3072

Dale Purves, G. J. A., David Fitzpatrick, William C. Hall, Anthony-Samuel LaMantia, James O. McNamara & Leonard E. White (2008). *Neuroscience*, Fourth ed., Sinaure Associates, Inc., Massachusetts, U. S. A.

DeCoursey, T. E. & V. V. Cherny (1997). Deuterium isotope effects on permeation and gating of proton channels in rat alveolar epithelium, *J Gen Physiol*, vol.109, No.4, pp415-434.

Goodman, R., C. A. Bassett, and A. S. Henderson (1983). Pulsing electromagnetic fields induce cellular transcription, *Science*, vol.220, No.4603, pp1283-1285.

Henderson, E. J., H. Nagano, H. Zalkin & L. H. Hwang (1970). The anthranilate synthetase-anthranilate 5-phosphoribosylpyrophosphate phosphoribosyltransferase aggregate. Purification of the aggregate and regulatory properties of anthranilate synthetase, *J Biol Chem*, vol.245, No.6, pp1416-1423.

Hermans, J., Jr., & H. A. Scheraga (1959). The thermally induced configurational change of ribonuclease in water and deuterium, *Biochim Biophys Acta*, vol.36, pp534-535.

Hill, R. S. & W. B. Rhoten (1983). Differential effects of microtubule-altering agents on beta-cells during development, *Am J Physiol*, vol.245, No.4, ppE391-400.

Hirakura, Y., T. Sugiyama, M. Takeda, M. Ikeda & T. Yoshioka (2011). Deuteration as a tool in investigating the role of protons in cell signaling, *Biochim Biophys Acta*, vol.1810, No.2, pp218-225.

Ikeda, M., Suzuki, M., Kishio, M., Hirono, M., Sugiyama, T., Matsuura, J., Suzuki, T., Sota, T., Allen, C.N., Konishi, S. & Yoshioka, T. (2004). Hydrogen-deuterium exchange effects on beta-endorphin release from AtT20 murine pituitary tumor cells, *Biophys J*, vol.86, No.1 Pt. 1, pp565-575.

Itoh, T. J. & H. Sato (1984). The effects of deuterium oxide (^2H_2O) on the polymerization of tubulin in vitro, *Biochim Biophys Acta*, vol.800, No.1, pp21-27.

Karasz, F.E. & Gajnos, G.E. (1976). Relative stability of the α-helix of deuterated poly(γ-benzyl-L-glutamate). *Biopolymers,* vol.15, pp1939-1950.

Katz, J. J. (1965). *Chemical and biological studies with deuterium*, 1-100 pp., Pennsylvania State University, University Park.

Kellenberger, E. (1991). The potential of cryofixation and freeze substitution: observations and theoretical considerations, *J Microsc*, vol.161, No. 2, pp183-203.

Klotz, I. M. (1962). *Water*, Academic Press, New York and London

Kushner, B. H., N. K. Cheung, K. Kramer, G. Heller, & S. C. Jhanwar (1998). Neuroblastoma and treatment-related myelodysplasia/leukemia: the Memorial Sloan-Kettering experience and a literature review, *J Clin Oncol*, vol. 16, No.12, pp3880-3889.

Landowne, D. (2000). Heavy water (D2O) alters the sodium channel gating current in squid giant axons, *Biol Bull*, vol.199, No.2, pp164-165.

Maeyama, K., R. J. Hohman, H. Metzger & M. A. Beaven (1986). Quantitative relationships between aggregation of IgE receptors, generation of intracellular signals, and histamine secretion in rat basophilic leukemia (2H3) cells. Enhanced responses with heavy water, *J Biol Chem*, vol.261, No.6, pp2583-2592.

Malaisse-Lagae, F., G. R. Brisson & W. J. Malaisse (1971). The stimulus-secretion coupling of glucose-induced insulin release. VI. Analogy between the insulinotropic mechanisms of sugars and amino acids, *Horm Metab Res*, vol.3, No.6, pp374-378.

Mandelkow, E. M. & E. Mandelkow (1992). Microtubule oscillations, *Cell Motil Cytoskeleton*, vol.22, No.4, pp235-244.

Marsland, D., L. G. Tilney & M. Hirshfield. (1971). Stabilizing effects of D2O on the microtubular components and needle-like form of heliozoan axopods: a pressure-temperature analysis. *Journal of Cellular Physiology*, vol.77, pp187-194.

Mentré, P. (1995). *L'eau dans la cellular* Masson, Paris.

Mentre, P. & G. Hui Bon Hoa (2001). Effects of high hydrostatic pressures on living cells: a consequence of the properties of macromolecules and macromolecule-associated water, *Int Rev Cytol*, vol.201, pp1-84.

Meves, H. (1974). The effect of holding potential on the asymmetry currents in squid giant axons, *J Physiol*, vol.243, No.3, pp847-867.

Montag, T. S., and A. Umanskii Iu (1976). Effect of combined use of antilymphocyte serum 7S antibodies and cyclophosphane on the growth of transplanted tumors in rats, *Fiziol Zh*, vol.22, No.5, pp630-634.

Omori, H., M. Kuroda, H. Naora, H. Takeda, Y. Nio, H. Otani & K. Tamura (1997). Deuterium oxide (heavy water) accelerates actin assembly in vitro and changes microfilament distribution in cultured cells, *Eur J Cell Biol*, vol.74, No.3, pp273-280.

Pilla, A. A., Kaufman, J. J. & Ryaby, J. T. (1987). *in Mechanistic Approaches to Interactions of Electric and Electromagnetic Fields with Living Systems* Blank, M. and Findl, E., ed., Plenum Press.

Proudhon, J. F., C. Almange, J. C. Palaric, J. Milon, P. Poulain & J. R. Giraud (1994). Left heart hypoplasia: diagnosis and current obstetrical management. Apropos of 4 cases, *J Gynecol Obstet Biol Reprod (Paris).* vol.23, No.4, pp425-431.

Rath, P., W. J. DeGrip & K. J. Rothschild (1998). Photoactivation of rhodopsin causes an increased hydrogen-deuterium exchange of buried peptide groups, *Biophys J*, vol.74, No.1, pp192-198.

Schauf, C. L. & J. O. Bullock (1980). Solvent substitution as a probe of channel gating in Myxicola. Differential effects of D2O on some components of membrane conductance, *Biophys J*, vol.30, No.2, pp295-305.

Schauf, C. L. & J. O. Bullock (1982). Solvent substitution as a probe of channel gating in Myxicola. Effects of D2O on kinetic properties of drugs that occlude channels, *Biophys J*, vol.37, No.2, pp441-452.

Scheraga, H. A. (1960). Helix-random coil transformations in deuterated macromolecules, *Ann N Y Acad Sci*, vol.84, pp608-616.

Sing, T.R. & J.L. Wood. 1969. Isotope effect on the hydrogen bound length. *J. Chem. Phys.* vol.50, pp3572-3576.

Sulowska, Z., & J. Wyczolkowska (1991). 5-Hydroxytryptamine releasing activity of the supernatants from cultured mouse spleen cells, *Arch Immunol Ther Exp (Warsz)*, vol.39, No.1-2, pp139-145.

Thomas, A. F. (1971). *Deuterium labeling in organic chemistry*, Appelton-Centry, New York.

Tsong, T. Y. (1989). Deciphering the language of cells, *Trends Biochem Sci*, vol.14, No.3, pp89-92.

Tsong, T. Y. & R. D. Astumian (1987). Electroconformational coupling and membrane protein function, *Prog Biophys Mol Biol*, vol.50, No.1, pp1-45.

Tuszynski, M. H. (2003). Gene therapy for neurological disease, *Expert Opin Biol Ther*, vol.3, No.5, pp815-828.

Urata, C., A. Watanabe, Y. Ogawa, N. Takei, H. Nomoto, M. Mizobe, Y. Abe, K. Mano & S. Urata (1989). Effect of deuterium oxide (D_2O) on the IgE-mediated Ca^{2+} influx, arachidonic acid and histamine release in rat basophilic leukemia. *Arerugi,* vol.38, pp285-295.

Watterson, J. G. (1991). The role of water in cell function, *Biofizika*, vol.36, No.1, pp5-30.

Zimmermann, A., H. U. Keller & H. Cottier (1988). Heavy water (D2O)-induced shape changes, movements and F-actin redistribution in human neutrophil granulocytes, *Eur J Cell Biol*, vol.47, No.2, pp320-326.
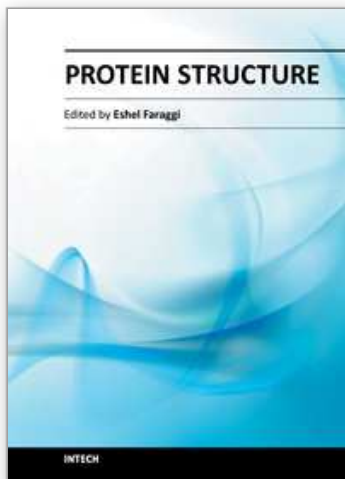
Protein Structure

Edited by Dr. Eshel Faraggi

Since the dawn of recorded history, and probably even before, men and women have been grasping at the mechanisms by which they themselves exist. Only relatively recently, did this grasp yield anything of substance, and only within the last several decades did the proteins play a pivotal role in this existence. In this expose on the topic of protein structure some of the current issues in this scientific field are discussed. The aim is that a non-expert can gain some appreciation for the intricacies involved, and in the current state of affairs. The expert meanwhile, we hope, can gain a deeper understanding of the topic.

How to reference

In order to correctly reference this scholarly work, feel free to copy and paste the following:

Takashi Sugiyama and Tohru Yoshioka (2012). Functional Difference Between Deuterated and Protonated Macromolecules, Protein Structure, Dr. Eshel Faraggi (Ed.), ISBN: 978-953-51-0555-8, InTech, Available from: http://www.intechopen.com/books/protein-structure/functional-difference-between-deuterated-and-protonated-macromolecules

INTECH
open science | open minds