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The dipole moment of membrane proteins: potassium channel protein and β-subunit

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Abstract

The mechanism of ion channel opening is one of the most fascinating problems in membrane biology. Based on phenomenological studies, early researchers suggested that the elementary process of ion channel opening may be the intramembrane charge movement or the orientation of dipolar proteins in the channel. In spite of the far reaching significance of these hypotheses, it has not been possible to formulate a comprehensive molecular theory for the mechanism of channel opening. This is because of the lack of the detailed knowledge on the structure of channel proteins. In recent years, however, the research on the structure of channel proteins made marked advances and, at present, we are beginning to have sufficient information on the structure of some of the channel proteins, e.g. potassium-channel protein and β-subunits. With these new information, we are now ready to have another look at the old hypothesis, in particular, the dipole moment of channel proteins being the voltage sensor for the opening and closing of ion channels. In this paper, the dipole moments of potassium channel protein and β-subunit, are calculated using X-ray diffraction data. A large dipole moment was found for β-subunits while the dipole moment of K-channel protein was found to be considerably smaller than that of β-subunits. These calculations were conducted as a preliminary study of the comprehensive research on the dipolar structure of channel proteins in excitable membranes, above all, sodium channel proteins.

Keywords: Calculation of dipole moment; K-channel protein; β-Subunit

1. Introduction

The participation of some protein molecules in nerve excitation have been inferred for some time. Although the progress of the research on the structure of membrane proteins has been slow, the number of articles on the analysis of the structure of purified membrane proteins has been increasing steadily in recent years [1–5]. Thus, the structure of the proteins in excitable membranes is beginning to be known in detail based on these analyses using X-ray and/or NMR techniques. These new developments, hopefully, render possible the formulaion of a new model of nerve excitation based on a molecular mechanism.

Unlike synthetic lipid bilayer membranes, nerve and muscle membranes consist of lipids and pro-

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teins. In general, the presence of protein molecules in biological membranes manifests itself as an increase in the electrical capacitance over that of simple lipid bilayer membranes. Furthermore, these additional capacitance due to the presence of dipolar proteins are usually frequency-dependent. For example, the electrical capacitance of nerve axon membrane decreases from a value of approximately 1 to 0.7–0.8 $\mu F/cm^2$ between 0.5 and 10 kHz. This is called dielectric relaxation, a phenomenon well known for synthetic and/or biological polymers in aqueous and/or non-aqueous solutions. Fig. 1a illustrates the frequency dependent capacitance of squid axon membrane observed by Takashima and Yantorno some years ago [6].

The frequency dependent capacitance is, in general, defined by Eq. (1) (see Boettcher et al. [7]).

$$C = C_\infty + \frac{\int (C_o - C_\infty)\phi(\tau)d\tau}{1 + j\omega\tau} \quad (1)$$

where C_o and C_∞ are the capacitances at limiting low and high frequencies (see Fig. 1a) $\omega = 2\pi f$ and τ is the relaxation time. $\phi(\tau)$ is the distribution function which dictates the spread of time contants τ.

If the same phenomenon is studied using time-domain techniques such as voltage clamp method, the presence of polar molecules manifests itself as a current spike immediately following the application of a pulse as shown by Fig. 1b. In this figure, I_∞ is an instantaneous current which flows through a simple lossless material such as lipid bilayer membranes. This current is defined by Eq. (2).

$$I_\infty = C_\infty (dV/dt) \quad (2)$$

I_d is a current which is due to a non-ideal or lossy material such as biological membranes. This current is formulated by Eq. (3) (see Oka et al. [8]).

$$I_d = (C_o - C_\infty) \int_{-\infty}^{t} \frac{dV(\tau)}{d\tau} \phi(t-\tau)d\tau \quad (3)$$

Although the results shown in Fig. 1a and 1b appear very different, they are, nevertheless, different expressions of the same phenomenon, i.e. the orientation of polar molecules in lipid bilayers and/or biological membranes.

The results shown by Fig. 1a and 1b are due to the presence of dipolar molecules in excitable and/or passive membranes regardless of the presence or absence of ionic channels. In other words, as long as there are some polar molecules in a membrane and there is certain degree of freedom of rotation, we are bound to observe a frequency dependent membrane capacitance and/or currents I_∞ and I_d as shown in Fig. 1a,b. However, there is some reason to believe that the presence of ionic channels in the membrane may enhance the appearance of these spike currents, particularly I_d, more than simple biological membranes without ionic channels. One of the reasons is that channel proteins have been believed to have a very large dipole moment.

As mentioned above, the results of X-ray crystallography or NMR analysis of natural as well as synthetic channel proteins or peptides have been published recently in various journals. In addition, the detail of these results are delineated in Protein Databases (website, http://www.rcsb.org/pdb/links.html). These databases can be transferred easily in its entirety to personal computers for the calculation of the dipole moments and for other analyses such as molecular dynamics. Using the X-ray databases obtained by Gulbis et al. [5] and Doyle et al. [2], the dipole moments of so-called β-subunit and K-channel protein were computed as the first step of these studies. As discussed later, one of these proteins, β-subunit were found to have a large dipole moment thus generating sufficient interests to speculate on the correlation between the orientation of dipolar proteins and the opening of ionic channels.

A brief review on the structure of sodium and potassium channel proteins in nerve membranes is given in the following.

1.1. Sodium channel protein

The voltage gated sodium channel protein is a large complex molecule consisting of α-subunits of 260 kDa and subunits of smaller sizes. However, the attempts to determine its structure with high resolutions have been frustrated so far [4] because

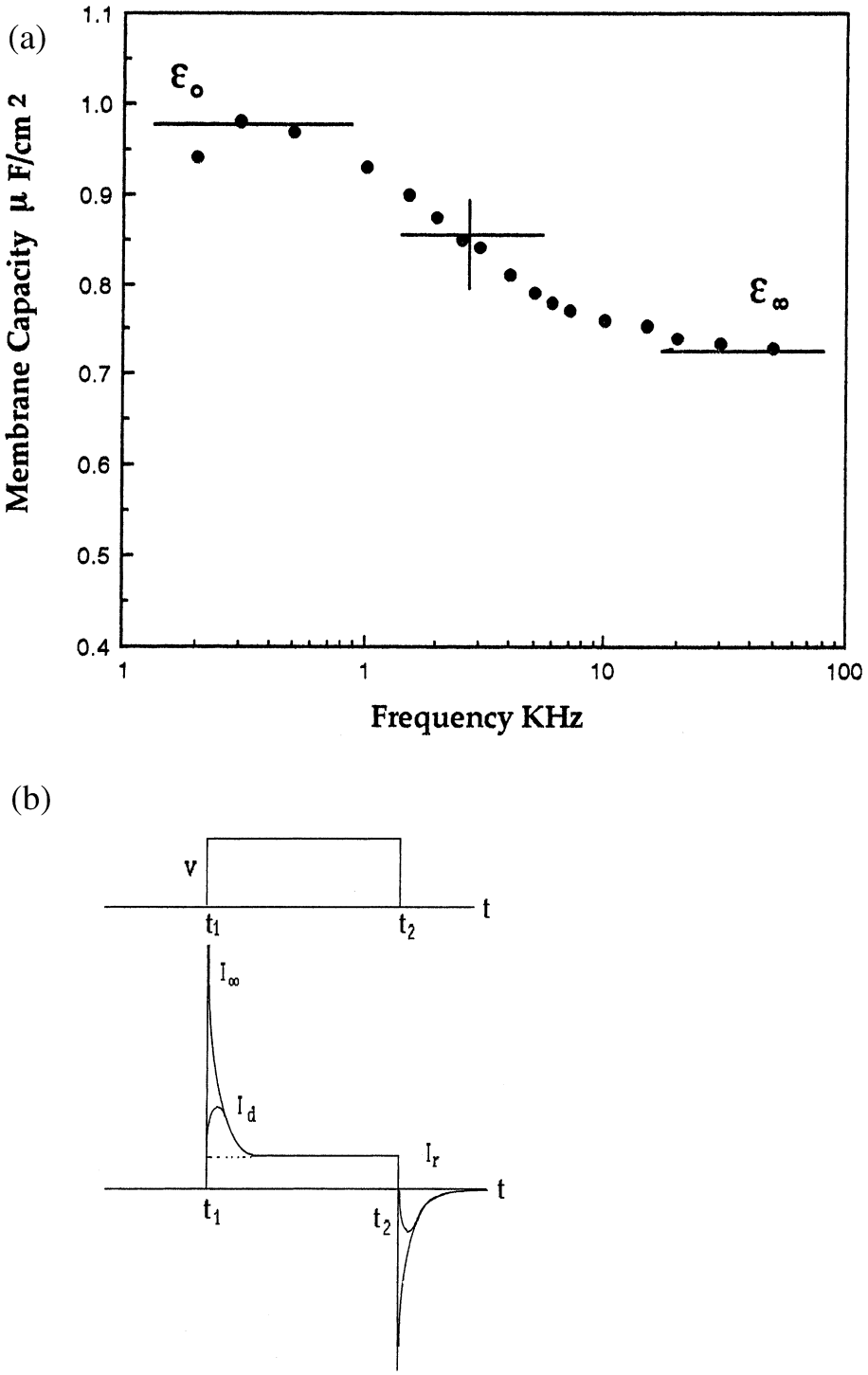

Fig. 1. (a) Frequency dependence of the squid axon membrane capacitance. The abscissa is frequency (kHz) and the ordinate is capacitance ($\mu F/cm^2$). (b) A displacement current schematically presented. The ordinate is current and the abscissa is time in ms. The explanation of I_∞ and I_d is given in the text. I_d is obtained by subtracting I_∞ from measured total displacement current. I_r is the ohmic current of the sample.

of its complexity, and thus, sodium channel protein is not listed as yet in the Protein Database, perhaps, because of insufficient information.

1.2. The structure of potassium-channel proteins and β-subunit

The structures of potassium channel protein (ID code 1BL8) and that of β-subunit (ID code 1QRQ) were investigated by Gulbis et al. [5] and Doyle et al. [2] in detail using X-ray crystallography. The β-subunit is formed by four so-called TIM-barrels (triose phosphate isomerase). These four TIM barrels form a permanent tetrameric complex. The channel forming four α-subunits are docked onto the plane of this tetramer.[1] In addition, a NADPH molecule is tightly bound to each TIM barrel.

The role of β-subunit complex is not precisely known at present, however, it was inferred by Gulbis et al. that β-subunit may be a voltage sensing aldo−keto reductase enzyme, without, however, elaborating the nature of the voltage sensor. This analogy is based on the structural similarity between these subunits and aldo−keto enzyme. Also the finding of a NADPH molecule tightly bound to each TIM barrel prompted this analogy. On the other hand, it has been believed by some researchers that the β-subunits play no role for the opening of K-channels.

Historically, the opening of ion channels in nerve membrane was attributed by Hodgkin and Huxley [9] either to the intramembrane movement of ionic charges or the reorientation of unidentified dipolar particles in the channel. In addition, the work by Tsong, Astumian and coworkers recognized the role of electric moments of membrane proteins for the voltage sensing mechanism of biological membranes (Tsong, [10] and Tsong and Astumian [11]). Much later than the work by Hodgkin et al., Armstrong and Bezanilla [12] and Meves et al. [13] found a spike current which is similar to the one depicted in Fig. 1b. This current was attributed by these investigators to the opening of sodium channels as the first step of ion conduction. As will be discussed later, however, there are other proteins in nerve membrane having a large dipole moment, e.g. β-subunits. These proteins can, likewise, produce a displacement current which is similar to the one reported. Under these circumstances, the displacement currents observed by Armstrong et al. may be produced by more than one type of channel or even non-channel proteins.

The present work aims at the computation of dipole moments of the tetrameric β-subunit as well as potassium channel proteins as the first step of this research. The full implication of these calculations will not be known, how ever, until the structures of other channel proteins, in particular, sodium channel protein, become known and their dipole moments are calculated.

2. Calculation of dipole moments

2.1. Core dipole moment

As is well known, the dipole moment of protein molecules consists of roughly two parts. (A) Core dipole moment and (B) surface charge dipole moment (Takashima and Asami [14], Takashima [15]). The core dipole moment arises mainly from polar side chains such as C=O (2.30 D) and N−H (1.31 D) bonds [16] (see Fig. 2). However, the present calculation is limited to C=O bonds only because of the inability of X-ray crystallography of detecting the coordinates of H atoms. The neglect of N−H bond moments may cause some errors in the final result of the core dipole moment. However, the magnitude of the core dipole moment is much smaller than that of surface charge dipole moment and the neglect of N−H bond moments would not cause a serious error in the final result. The polar bonds in the backbone chain were mostly ignored because of their small values except C−N bonds.

2.2. Surface charge dipole moments

The dipole moment due to surface charges is much greater than the core dipole moment and is, by far, the most important component of the dipole

[1] The structure of α-subunits were not studied in detail by Gulbis et al. and it is not clear whether α-subunits in their study are the same protein as the K-channel protein (1BL8) studied by Doyle et al.

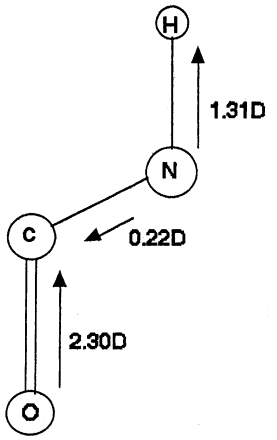

Fig. 2. The vectorial relation in the peptide group and the dipole moments of C=O and N–H bonds [16]. Because of the reason explained in the text, the dipole moment of N–H group is not included for the calculation of the core dipole moment.

moments of protein molecules. However, the calculation of the surface charge dipole moment of protein molecules is not necessarily straightforward even for water soluble cytoplasmic proteins such as lysozyme and myoglobin. Calculation of the dipole moment of water-insoluble membrane proteins is even more cumbersome as will be discussed later.

In general, in order to calculate surface charge dipole moments, the state of ionization of polar groups such as COO^- or N^+H_3 must be known as accurately as possible. The difficulties arise from (A) the shift of ionization constant of polar sites from the intrinsic p*K* values due to electrostatic interactions among neighboring charges (Antosiewicz and Porschke [16]). This problem, however, can be alleviated, if not satisfactorily, using the theory by Tanford and Kirkwood [17] with the modifications by Warshel and Russel [18]. (B) For water soluble cytoplasmic proteins, polar groups are mostly exposed to the surface of protein molecules. However, membrane proteins are water insoluble and polar groups are often buried in the interior of proteins. Even if some of these polar groups are located on the surface of protein molecules they are often surrounded by lipid environments thus making no or very little contacts with external aqueous environments. This is a very different feature of membrane proteins compared to water soluble cytoplasmic proteins.

The polar groups which are buried in lipid environments are not ionized and, accordingly, do not contribute to the surface charge dipole moment. Thus, use of these unionized polar groups for the calculation of dipole moment will cause a large error for the value of surface charge dipole moments. Thus, it is extremely important to find those hidden unionized polar sites carefully and eliminate them from the computation as much as possible.

At present, however, there is no theoretical method to implement this correction and the only way for the removal of hidden unionized polar sites is to use visual inspections. The procedure is (1) plot the protein molecule in two-dimensional coordinates, e.g. $X-Y$ plane. (2) All the polar groups at the protein–lipid interfaces and also those buried inside the protein moiety are eliminated. This leaves us only with the polar groups at the interfaces between protein surface and cytoplasmic and external aqueous phases. (3) The removal of buried polar groups is repeated by rotating the protein molecule onto other planes, i.e. $Y-Z$ and $Z-X$ plane and the search of buried polar sites is repeated.

As discussed later, the removal of buried polar groups results in a significant increase in the calculated dipole moment namely from approximately 800 to 1300 DU. However, because of the subjective nature of this procedure, admittedly, the calculation of the dipole moment of membrane proteins cannot be as accurate as for water soluble cytoplasmic proteins.

Fig. 3 illustrates the structure of tetrameric β-subunit complex plotted on the $Z-X$ plane without showing the distribution of surface charges. The arrows shown on each TIM barrel (A, B, C and D) illustrate the dipole moment vectors calculated for each TIM barrel (see below for the detail of calculations). Likewise, Fig. 4 shows the lateral view of a β-sununit projected on $X-Y$ plane. The crosses and circles are positive and negative surface charges.

Fig. 5 shows the structure and surface charge distribution of K-channel protein. The crosses and circles are positive and negative surface charges

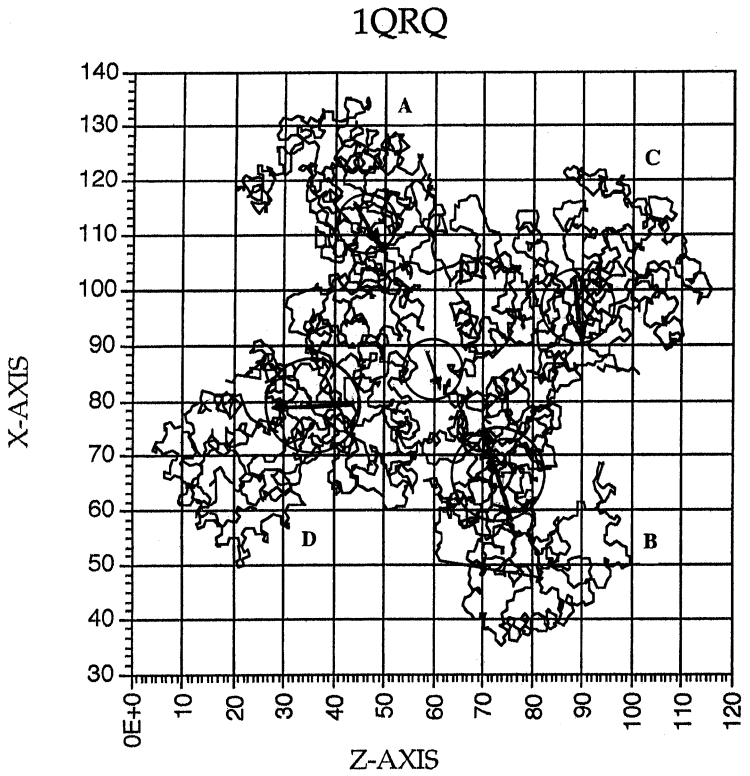

Fig. 3. Two-dimensional plot of β-subunit in the $Z-X$ plane without α-particles and DPNH molecules. The arrows with circles are the dipole moment calculated for each TIM barrel. The arrow at the center is the vectorial sum of the dipoles A–D. The y-axis is taken to be along the transmembrane component.

(buried charges are eliminated as for β-subunits). The charge density of K-channel protein seems to be much smaller than that in β-subunits indicating a smaller dipole moment for this protein than that of β-subunits. The method of calculation of the dipole moment is discussed in detail below.

2.3. Method of the calculation of surface charge dipole moment

The three-dimensional coordinates (the resolution of 2.8 Å) of all the atoms including charged sites were obtained from the protein databank (RCSB.ORG/PDB/links.html)). The ID code of β-subunits is 1QRQ and 1BL8 for K-channel protein. In this work, the X, Y and Z components of the dipole moment were calculated separately and the net dipole moment was reconstituted vectorially using the following formulae:

$$\mu^2 = \mu_x^2 + \mu_y^2 + \mu_z^2. \quad (4)$$

The x-, y- and z-components are defined by

$$\mu_x = \sum n_j \cdot e \cdot (X^+ - X^-) \quad (5)$$

where n_j is the number of surface charges, e is elementary charge. X^+ and X^- are the positive and negative charge centers and are defined by

$$X^+ = \sum (L_j^+ \cdot X_j) \quad \text{and} \quad X^- = \sum (L_j^- \cdot X_j). \quad (6)$$

Likewise, similar equations hold for Y^+ and Z^+ components. X_j, Y_j and Z_j are the x, y and z coordinates of these charges and can be found in the database. Moreover, L_j^+ and L_j^- are given by the Henderson–Hasselbalch equation (Bray and White [19]), i.e.

$$L_j^- = B/(1 + B) \quad \text{for Asp, Glu, Tyr and C terminal.} \quad (7)$$

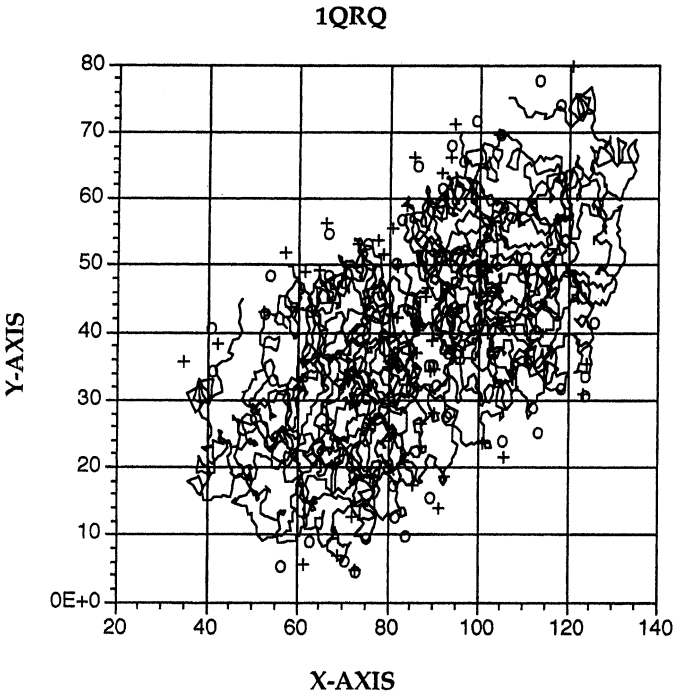

Fig. 4. Lateral view of β-subunit plotted on the $X-Y$ plane. Crosses and circles are positive and negative charges which are exposed to the aqueous external solution and internal cytoplasmic medium.

$$L_j^+ = B/(1 + B) \quad \text{for Lys, Arg, His and N-terminal.} \tag{8}$$

where

$$B = 10^{\mathrm{pH}-\mathrm{p}K}. \tag{9}$$

In this equation pK is either intrinsic pK or those corrected for electrostatic interactions using Kirkwood and Tanford theory. It is known that the use of corrected pK values does improve the results in some cases [20]. However, the calculation of the pK shifts is cumbersome and time consuming. In addition, the polar sites which are buried in lipid moieties and can be overlooked would cause much greater errors for the calculation of dipole moments than the pK shifts. Under these circumstances, it was decided to use intrinsic pK values for all the polar sites.

The basic polar amino acid groups used for the calculation of dipole moment are Lys, Arg, histidine and N-terminal. The acidic amino acids are Asp, Glu, tyrosine and C-terminal. As mentioned before, membrane proteins are embedded in lipid bilayers and only some portion of these proteins are exposed to either cytoplasmic side or external fluids (see Figs. 4 and 5).

Usually, Eq. (5) is used for the calculation of the dipole moment at the isoelectric point where

$$\sum n_j \cdot L_j^+ = \sum n_j \cdot L_j^- \tag{10}$$

where n_j is the number of positive and negative charges and L_j^+ and L_j^- are defined by Eqs. (7) and (8).

3. Results of the numerical calculation

The calculation of dipole moment using the database was performed by parts for each TIM barrel and then the total dipole moment of β-subunit is calculated by vectorially summing the dipole moments of TIM barrels. The results are tabulated in Table 1. Note the large dipole moment

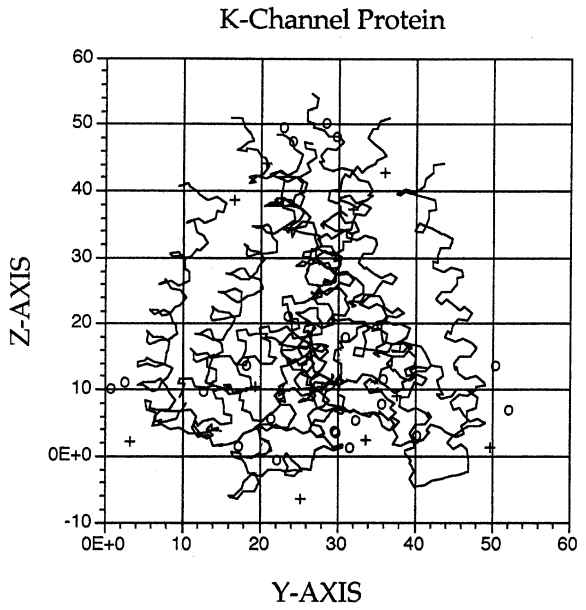

Fig. 5. The two-dimensional plot of K-channel protein with surface charges ('x' positive charges and 'o' negative charges). *Z*-axis is taken to be along the transmembrane axis.

along the *y*-axis. This means that the tetrameric β-subunit has a large dipole moment perpendicular to the plane of tetramer, i.e. Z–*X* plane (see Fig. 3).

The dipole moment of K-channel protein was calculated separately using the same method and the results are shown in Table 1. It should be noted that K-channel protein alone, not like β-subunits, does not have a large dipole moment. (Note that the *Y*-axis is chosen to be the transmembrane direction.)

As discussed before, squid axon membrane exhibits a frequency dependent capacitance with a center frequency of about 2 kHz (see Fig. 1a). As already discussed, the TIM barrels of β-subunits, was found to have a large dipole moment. On the other hand, K-channel protein has only a dipole moment of moderate magnitude. This clearly suggests that β-subunits, rather than K-channel protein, can be one of the sources of the frequency dependent membrane capacitance and also the displacement current observed by various investigators.

In general, the relaxation time of rotation of dipolar molecules is given by a simple equation [21] as shown by Eq. (11).

$$\tau = 4\pi\eta abc/kT \tag{11}$$

where *a*, *b* and *c* are the major and minor axes of the protein, η is the viscosity of cell membrane in centipoise, *k* is the Boltzman constant. For the present calculation, the major and minor axes of tetramer *a*, *b* and *c* are approximately 100, 100 and 40 Å. Although the viscosity of biological membrane is not accurately known, particularly in or near ionic channels, it may not be unreasonable to assume the viscosity of biological membranes to be about several times that of water, i.e. about 10–11 centipoises (equivalent to the viscosity of stearic acid at 25–50 °C according to the handbook of Chemistry and Physics). Using these in Eq. (11), we obtain $\tau = 6.06\times10^{-4}$ s or the characteristic frequency ($f_r = 1/2\pi\tau$) of about 200–300 Hz, a value much lower than that shown in Fig. 1, i.e. 2 kHz. This result strongly suggests that the frequency dependent membrane capacitance is not due to the orientation of the entire teteramer of β-subunits but rather due to the tethered orientation of individual TIM barrels.

In order to confirm that TIM barrels are responsible individually for the frequency dependent membrane capacitance shown in Fig. 1a, we can again use Eq. (11) in order to calculate the characteristic frequency of orientation for each TIM barrels using $a = 40$ Å, $b = 30$ Å and $c = 30$

Table 1
The dipole moments of β-subunits (TIM barrels)

	X	*Y*	*Z*	Total
Dipole moment of TIM barrels[a]				
A	169.837	−286.835	146.288	364.031
B	−271.455	−423.062	21.103	503.105
C	55.836	−443.083	−445.165	630.565
D	198.526	−164.948	−79.387	270.042
Total	152.744	−1317.92	−357.161	1373.98
Dipole moment of K-channel protein[b]				
Total	−378.401	−104.960	−400.777	561.093

The unit of dipole moment is Debye unit (3.33×10^{-30} C m).

[a] *Y*-axis is taken to be along the transmembrane direction.

[b] *Z*-axis is taken to be along the transmembrane direction.

Å. The relaxation time was found to be $\tau = 7.3\times10^{-5}$ s or the characteristic frequency $f_r = 2.180$ kHz. This value is sufficiently close to that obtained from Fig. 1, i.e. about 2.5 kHz. Having obtained these results, it may be reasonable to assume that the basic unit of dipolar orientation observed by Takashima and Yamtorno is the TIM barrel rather than the entire tetrameric β-subunits. On the other hand, a similar calculation indicates that the relaxation frequency of K-channel protein with a dimension of approximately $40\times40\times50$ A^3 is approximately 0.7–0.8 kHz, a value considerably lower than that shown in Fig. 1.

4. Discussion

Using the X-ray database, the dipole moments of β-subunit and K-channel protein are calculated numerically. The dipole moment of each TIM barrel is found to be approximately 300–400 DU. The vectorial summation of the dipole moments of TIM barrels, as shown in Table 1, turns out to be approximately 1400 DU. This proves that TIM barrels which constitute the basic component of β-subunit indeed have a large dipole moment. In addition, the relaxation frequency of TIM barrel having a dimension of approximately $40\times30\times30$ $Å^3$ was found to be approximately 2.0–2.2 kHz (see Fig. 1a). This value is very close to the characteristic frequency observed for the frequency dependent membrane capacitance of squid axon membrane, i.e. 2.5 kHz. These two observations indicate that the TIM barrels in β-subunit may indeed be one of the proteins that gives rise to the observed frequency dependent membrane capacitance in squid axon membrane. The significance of this finding is yet to be found by further investigations.

This work is trying to explore the hypothesis that the orientation of channel proteins may be the first step of channel opening. As the first step of this endeavour, the dipole moments of β-subunits and potassium channel protein were calculated. The structure of sodium channel protein is not fully elucidated at present and its dipole moment cannot be computed presently. As shown in Table 1, β-subunit was found to have a large dipole moment across the membrane. On the other hand, the dipole moment of K-channel protein is much smaller than β-subunit contrary to our expectation.

This paper does not offer an explanation why the structure of β-subunit is similar to aldo–keto enzyme and also why NADPH molecule is tightly attached to each TIM molecule. Nevertheless, the basic concept used in this work, i.e. the voltage sensor may be a dipolar molecule is compatible with the classic concept proposed qualitatively by Hodgkin and Huxley. Although K-channel protein alone was not found to have a large dipole moment, β-subunit which is believed to be coupled to K-channel protein has a large transmembrane dipole moment. Although this observation indicates that β-subunit is the voltage sensor for potassium channels, the result does not offer a conclusive evidence as yet.

A similar analysis on the dipole moment of sodium channel protein will be carried out whenever its structure is fully elucidated.

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