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The dipole moments of membrane proteins: potassium channel proteins. II. T1 assembly. Search for the voltage sensor

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Abstract

The dipole moments of potassium channel protein (Kcsa) and β-subunits were discussed in the previous paper of this series [Takashima, Biophys. Chem. 94 (2001) 209–218]. While the dipole moment of β-subunits was found to be very large, the dipole moment of Kcsa turned out to be somewhat smaller than β-subunits. As the continuation of this work, the discussion of the present paper is focussed on the dipole moment of T1 assembly, another component of the K-channel. As discussed later, the calculation using the X-ray crystallographic data by MacKinnon et al., revealed an astoundingly large dipole moment for T1 assembly. The dipole moment of T1 assembly combined with the likewise large dipole moment of β-subunits amounts to a sufficient value to play an essential role as a voltage sensor of potassium channel.

Keywords: Calculation of dipole moments, Kcsa, T1 particle, β-subunits of K-channel

1. Introduction

The main focus of the research on nerve excitation has been the analysis of Na and K ion fluxes [1–5] using voltage clamp method combined with other techniques. One of the most fascinating results out of these studies is the finding of ion channels and the unique specificity of the passage of Na and K ions through them. In spite of the accumulation of enormous amount of information with regard to the mechanism of ion fluxes through these channels, the detailed knowledge on the structure of ion channels or the nature of channel proteins is still incomplete. In addition, although the opening and/or closing of ionic channels are highly voltage sensitive, the identity of the voltage sensor in excitable membranes or ionic channels is still largely unknown at present.

In general, the presence of proteins, either channel and/or non-channel proteins, in lipid bilayer membranes can be detected, although only qualitatively, by electro-physiological methods (Armstrong and Bezzanilla [4] and Meves [5]). However, the identification and/or the detailed analysis of the structure of these membrane proteins are nearly impossible by these in vivo techniques. At present, the emphasis of the research

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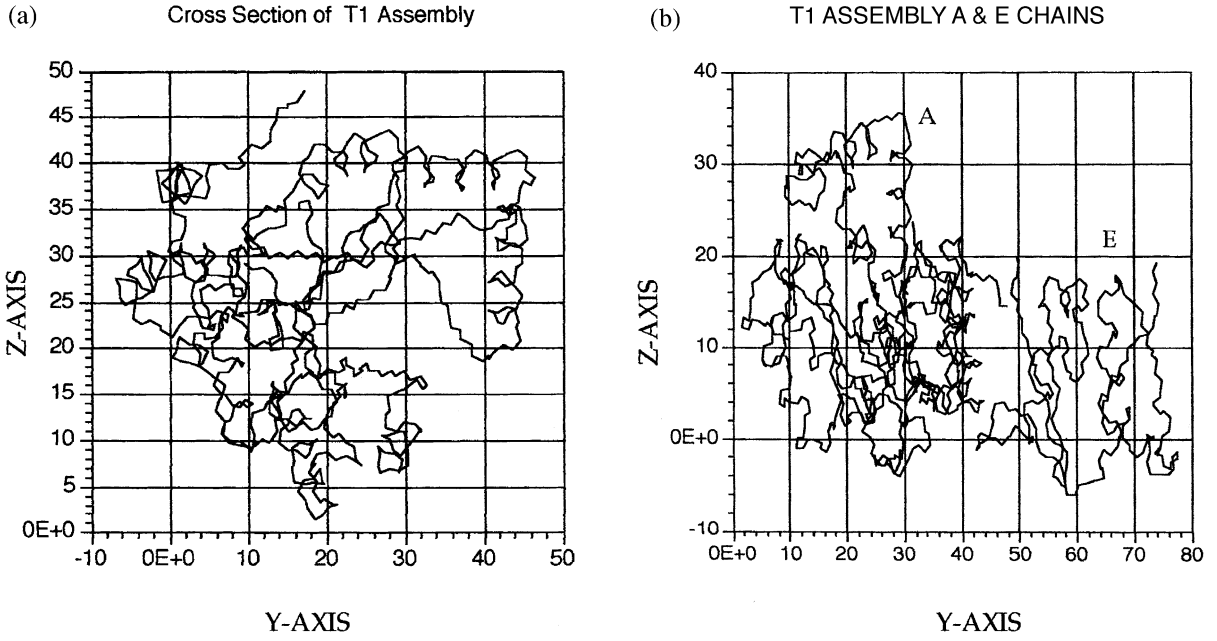

Fig. 1. Two-dimensional projections of T1 particles. (a) Vertical view and (b) lateral view. The symbols × and ○ are the positive and negative charges on side chains. The charges are used for the calculation of dipole moments.

on excitable membrane is, shifting to the detailed study of the structure of ion channel and/or channel proteins using, for example, X-ray crystallography. Some of the examples are the X-ray crystallographic studies of K-channel proteins by Gulbis et al. [6,9], Doyle et al. [7] and by Doak et al. [8] on sodium channel proteins. Of these, the outstanding research by MacKinnon's group on K-channel proteins is in particular worthy of note. The results of the X-ray studies on the structure of K-channel proteins can perhaps be summarized as follows. (A) K-channels consist of three particles, i.e. (a) K-channel protein (Kcsa), (b) β-subunit and (c) T1 assembly.

The main purpose of the present work is to investigate the nature and magnitude of the dipole moment of T1 assembly. Combining this result with the dipole moment of β-subunits, it was hoped that the possible mechanism of the voltage sensing in potassium channels could be identified. It is already known that β-subunits have a very large dipole moment while K-channal protein Kcsa has only a small dipole moment [12]. In order to reinforce the above-mentioned hypothesis that dipole moment may play an important role for the voltage sensing in ionic channels, it is essential to study the magnitude of the dipole moment of all the components in ionic channels in order to ascertain that at least some of the channel components have a significant dipole moment.

As already mentioned, the dipole moments of β-subunits and Kcsa have been investigated and they were found to have a large dipole moment. Unless there is another component yet to be found, T1 assembly is, at present, the only major component with unknown dipole moment in K-channel.

2. The structure of T1 particles

The structure of T1 particle is shown in Fig. 1a and b, Fig. 1a illustrates the projection of T1 particle in $Y–Z$ plane and Fig. 1b shows the projection in $X–Y$ plane. The dipole moment of charged protein molecules consists of two components, i.e. (1) surface charge dipole moment and

(2) core dipole moment arising from polar bonds such as C=O and N–H bonds.

First of all, the method of dipole moment calculation is briefly discussed as shown below.

2.1. Method of the calculation of surface charge dipole moments

The three-dimensional coordinates of all the atoms of channel proteins were found in the protein databank (RCSB.Org.PDB). The ID codes of β-subunits are 1QRQ and 1BL8 for K-channel protein. In addition, the code for T1 assembly is 1EXB.

2.2. Core dipole moment

As mentioned, the dipole moment of protein molecules consists of two parts: (A) core dipole moment and (B) surface charge dipole moment. However, the dipole moment of N–H bonds cannot be calculated with a database obtained with X-ray crystallography. This is because of the inability of X-ray crystallography to detect the coordinates of H atoms because of its low electron density. Therefore, only the dipole moments of CO bonds are computed in this study. The omission of N–H bond dipole moments, needless to say, causes some errors in the magnitude of total dipole moment of protein molecule. Core dipole moments, however, are much smaller than the surface charge dipole moment and are only a minor component of the total dipole moments of proteins. Thus, the omission of N–H bond moments actually causes only a small error in the calculated total dipole moment of a protein.

2.3. Calculation of surface charge dipole moment

First of all, the method of calculation of the surface charge dipole moment is discussed as follows. The *X*, *Y* and *Z* components of the dipole moment were calculated separately and the net dipole moment was obtained by adding them vectorially using the following formula:

$$\mu^2 = \mu_x^2 + \mu_y^2 + \mu_z^2 \tag{1}$$

The μ_x, μ_y and μ_z are given by

$$\mu_x = \sum n_j e(X^+ - X^-) \tag{2}$$

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

$$X^+ = \sum (L_j^+ X_j) \text{ and } X^- = \sum (L_j^- X_j) \tag{3}$$

Likewise, similar equations hold for Y and Z components. X_j, Y_j and Z_j are the x, y and z coordinates of charges and can be found in the database. Morever, L_j^+ and L_j^- are defined by the following equations:

$L_j^- = 1/(1+B)$ for Asp, Glu, Tyr and C-terminal

$L_j^+ = B/(1+B)$ for Lys, Arg, His and N-terminal

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

In this equation, pK is either intrinsic pK or the pK value corrected for the electrostatic interaction using Kirkwood and Tanford theory [10]. In this work, intrinsic pKs were used exclusively without the cumbersome Kirkwood–Tanford correction because of the extraordinary magnitude of the dipole moment of channel proteins. Under these conditions, the use of intrinsic pK without the Kirkwood and Tanford correction is well justified. The calculation of dipole moments were carried out at isoelectric point where the following equation holds,

$$\sum n_j L_j^+ = \sum n_j L_j^- \tag{4}$$

2.4. Reliability of the numerical calculation of dipole moments using protein databases

The dipole moments of various globular proteins calculated using protein databases are shown in Table 1 along with those measured. As shown, calculated dipole moments are in reasonable agreements with those measured. However, these calculations also show some deviations from those

Table 1
Comparison of the calculated and measured dipole moments of some globular proteins

	Surface	Core	⟨θ⟩	Total	Exp.
Myoglobin	247	57	148	199	170[a]
RnaseA	344	24	46	361	336[b]
Cytochrome *C*	267	49	138	233	235[c]
Lysozyme	127	61	93	138	122[d]
Phospholipase	130	34.0	104	125	141[d]

Unit of dipole moment: Debye Unit (3.33×10^{-30} C m).
[a] P. Schlecht, Biopolymers 8 1969; 757–765.
[b] S. Keef, E.H. Grant, Phys. Med. Biol. 19(5) 1974; 701–707.
[c] S. Takashima, K. Asami, Biopolymers 33 1993; 59–68.
[d] S. Takashima, Biophys. J. 64 1993; 1550–1558.

measured, particularly, for proteins with large molecular weights.

3. Results of the calculation

3.1. Dipole moment of T1 particles

The results of the calculation obtained with T1 particle (1EXB) are summarized in Table 2. T1 particle consists of two particles, i.e. 1EXA and 1EXE. There are two ways to calculate the dipole moments of T1 particle, i.e. (a) the dipole moments of 1EXA and 1EXE particles are calculated separately and the dipole moments of two components are summed vectorially as shown in Table 2. $A+B$ indicates the sum of the dipole moments of the particles A and B separately calculated. $(A+B)$ is calculated considering 1EXA and 1EXB together as a dimer. Note that the *Z*-component of the calculated dipole moment is extraordinarily large in both calculations. This means that the T1 particle has a large dipole

Table 2
The dipole moment (DU) of T1 particles 1EXA and 1EXE

	XDP	YDP	ZDP	Total
Charge $A+E$[a]	−377.60	−548.95	−2110.24	2113.0
Core $A+E$	99.57	12.39	10.71	
(A+E)	−278.03	−536.55	−2099.53	2183.7
Charge $(A+E)$[b]	−215.71	−943.84	−2407.17	2594.5
Core $(A+E)$	99.57	12.39	10.71	
Charge+core	−116.14	−931.46	−2396.46	2573.7

[a] The dipole moments of A and E are calculated separately and they are summed vectorially.
[b] The dipole moments of A and E are calculated all together as a dimer.

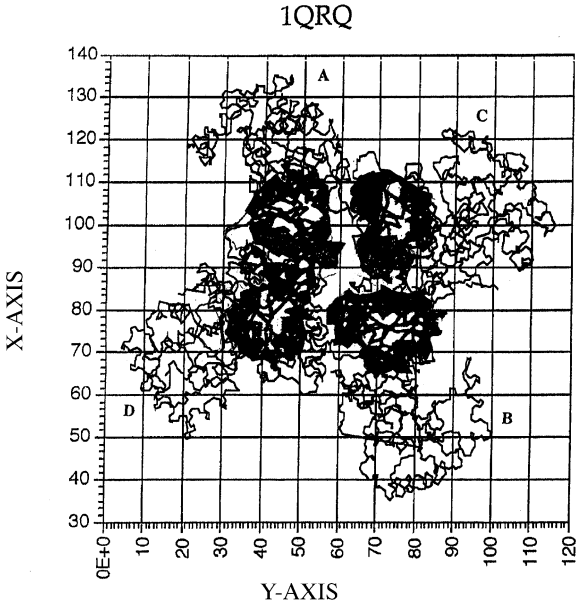

Fig. 2. The presentation of β-subunit plane and four T1 particles docked on β-subunit plane. The four NADP molecules are omitted.

Table 3a
The dipole moments of β-subunits surface charge+core dipole moments (DU)

	XDP	YDP	ZDP	Total
A	169.83	−286.83	146.28	364.03
B	−271.45	−423.06	21.10	503.10
C	55.836	−443.08	−445.16	630.56
D	198.52	−164.94	−79.16	270.04
Total	152.744	−1317.92	−357.16	1373.9

Table 3b
The sum of the dipole moments of β-subunits and T1 particles

	XDP	YDP	ZDP	Total
β-subunit	152.7	−1327.9	−357.16	
T1 particle	−116.74	−9.314	−2396.4	
Sum	36.6	−1337.2[a]	−2753.5	3061.4

[a] The total dipole moment of T1 particle in *Y*-axis is 1337×4 D.

Table 4
The dipole moments (DU) of K-channel protein Kcsa (1BL8) and the result with Kcsa with a K-channel blocker TBA

	XDP	YDP	ZDP	Total
1BL8	1382.7	415.7	472	1519.1
1JVM[a]	548.0	98.07	470.7	729.2

[a] 1BL8 with K-channel blocker TBA and Rb ion.

moment along the direction of K-channels or perpendicular to the plane of β-subunit.

The calculated dipole moments of β-subunits are also shown below in order to facilitate the comparison of the unusual magnitude of the dipole moment of T1 complex with those of other particles in K-channel. The geometry of tetrameric β-subunit and four T1 particles is depicted in Fig. 2. In short, the planar β-subunit assembly is docked on four T1 particles with their *Z*-axes perpendicular to the plane of β-subunits. Because of the fourfold symmetry, the x- and *y*-components of the dipole moment of T1 complexes will perhaps reduce to a small value. However, the *Z*-axes of four T1 particles are nearly parallel to one another so that the large dipole moments of four T1 particles sum up to be an exceedingly large net dipole moment of $2407 \times 4 = 9608$ D along the *Z*-axis, in other words, parallel to the K-channel pore.

3.2. The dipole moment of K-channel protein with and without channel blocker

As has been discussed, β-subunits (TIM barrels) and T1 assembly have an exceedingly large dipole moment. Although, a rather small dipole moment was found in the previous calculation for Kcsa, the calculation was repeated with a great care in order to eliminate the possible errors in the previous calculation. The recalculation found a much larger dipole moment of 1726 DU for the K-channel protein. With this new result, the role of 1BL8 was reinterpreted accordingly.

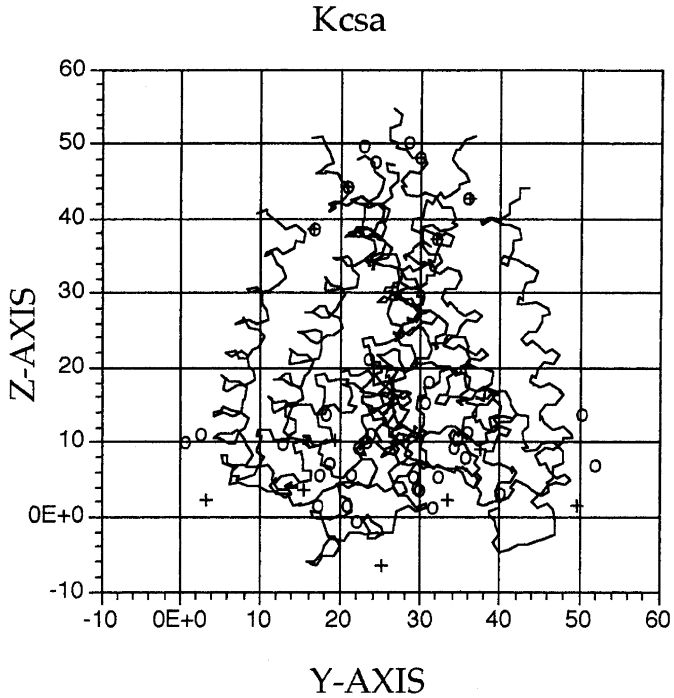

Fig. 3. Two-dimensional presentation of Kcsa in *Y–Z* plane. The symbols × and ○ are positive and negative surface charges on side chains (omitted).

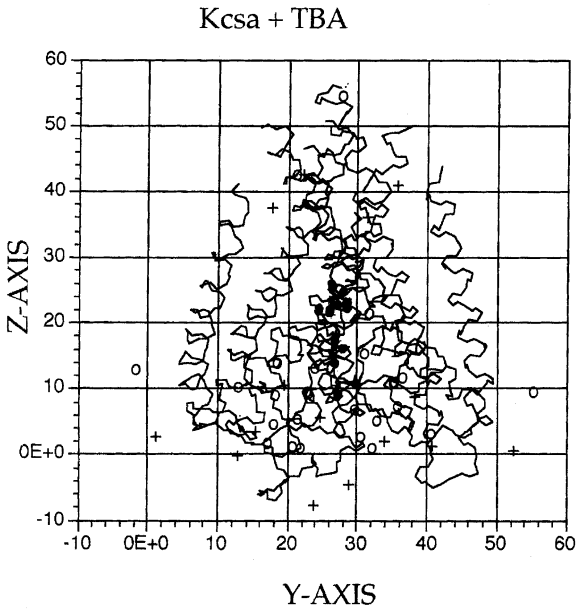

Fig. 4. Two-dimensional presentation of Kcsa in *Y–Z* plane with the addition of TBA and Rb. The significance of × and ○ is the same as in Fig. 4. The dark shaded object is TBA. Note considerable alteration of the coordinates of surface charges although the overall conformation of channel protein is not markedly altered.

Tables 3 and 4 list the dipole moment of 1BL8 with the addition of potassium channel blocker tetrabutyl ammonium (TBA) and Rb. As shown in this table, the dipole moment of 1BL8 was found to decrease drastically to a smaller value of approximately 730 DU. The conformation of 1BL8 and that of 1JVM are illustrated in Figs. 3 and 4. It should be noted that the addition of TBA does not alter the backbone chain of 1BL8 drastically. However, the distributions of both positive and negative surface charges are altered significantly.

In any case, the large dipole moment of 1BL8 seems to be related to the opening of K-channels and the decrease of dipole moment by channel-blocker seems to interfere the function of K-channel protein.

4. Discussion

This series of papers started as an endeavour to investigate the dipole moments of K-channel proteins using the X-ray data obtained by Gulbis et al. (see Ref. [6]). The dipole moments of K-channel proteins such as Kcsa, β-subunits and T1 assembly have been calculated by the present author. First of all, the dipole moment of Kcsa turned out to be the smallest among the above-mentioned three channel proteins. Because of this observation, K-channel protein, Kcsa could not be classified as the primary voltage sensor to initiate the opening of K-channels. In other words, the initial step of K-channel opening may not be the response of Kcsa particle to the applied electrical pulse. While the dipole moment of β-subunits was found very large, the dipole moment of T1 assembly turned out to be even larger.

Furthermore, if the binding of four T1 particles with β-subunit is mutually parallel and perpendicular to the plane of β-subunit, the T1 particles–β-subunit assembly results in an astoundingly large net dipole moment. Close inspection of the structure of T1 particles, particularly with respect to the location of E-particle clearly shows the role of E-particles for the binding T1 proteins onto the plane of β-subunits. This indicates that the orientation of the T1 particles onto the plane of β-subunit is not arbitrary but E-particles seem to make a direct contact with β-subunit plane. With this observation, it was concluded that the mutual orientation of four T1 particles is always parallel resulting in a very large net dipole moment.

Participation of the dipole moment in nerve excitation has long been considered by some investigators, namely Hodgkin and Huxley [11], and Tsong and Astumian [12] to name but a few. Since the electrical pulse is applied across the membrane or along the K-channel pore, the dipole vector of polar molecules such as T1 particle and β-subunits will respond in such a way to reorient their dipoles following the law dictated by the Langevin equation [13] (Fig. 5).

$$\langle \cos\theta \rangle = L(\mu E/kT) = L(x) = (X/3) - \left(X^3/45\right) + \ldots \quad (5)$$

where $L(\mu E/kT)$ is the Langevin function and E is the intensity of electrical field in the membrane. $\langle \cos\theta \rangle$ indicates the mean orientation of dipole vectors as a function of the relative intensity of

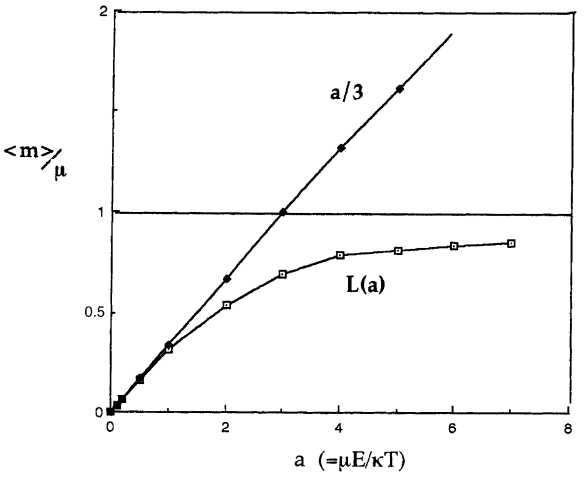

Fig. 5. The Langevin function $L(a)$ depicting the time course of the increase of electric polarization. The straight line is the limiting linear approximation with a slope of $a/3$.

electrical field in the pore. The value of direction cosine $\langle \cos \theta \rangle$ changes as a function of either m or E or both.

In this equation, the relative orientation of a dipole increases as a function of $x = \mu E/kT$ and eventually reaches an equilibrium orientation. In other words, the slope of increase and also the maximum extent of dipole orientation depend on the magnitude of dipole moment and also the strength of electrical fields. This simple theory can readily be applied to the opening of a channel. Namely, the extent of channel opening is dictated nearly the same way although the calculation of the effective field strength in the pore may be considerably different from those in homogeneous solution because of the spatial constraints inside small pores.

In view of these analyses, one may be able to conclude that the T1 assembly may be the voltage sensor of K-channels. The role of β-subunit may be to provide T1 particles with a platform or vice versa. As has been discussed, β-subunit has a dipole moment nearly as large as that of T1 assembly. However, the β-subunits have a large dipole moment along *Y*-axis while T1 assembly has a large dipole moment along *Z*-axis. The interpretation of this observation may not be straightforward.

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