GIBBS-DONNAN RATIO AND CHANNEL CONDUCTANCE OF *TETRAHYMENA* CILIA IN MIXED SOLUTION OF K^+ AND Ca^{2+}

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ABSTRACT A single cation-channel from *Tetrahymena* cilia was incorporated into planar lipid bilayers. This channel was voltage-independent and is permeable to K^+ and Ca^{2+}. In the experiments with mixed solutions where the concentrations of K^+ and Ca^{2+} were varied, the single-channel conductance was found to be influenced by the Gibbs-Donnan ratio. The data are explained by assuming that the binding sites of this channel were always occupied by two potassium ions or one calcium ion under the present experimental conditions (5 mM – 90 mM K^+ and 0.5 mM – 35 mM Ca^{2+}) and these bound cations determined the channel conductivity.

INTRODUCTION

The swimming behavior of ciliate protozoa such as *Tetrahymena* and *Paramecium* is related to the membrane potential which is controlled by several ions, especially K^+ and Ca^{2+} (Machemer and Ogura, 1979; Kung and Saimi, 1982; Connolly and Kerkut, 1981; Onimaru et al., 1980). The backward swimming is triggered by the action potential which is associated with the influx of Ca^{2+}. The importance of accumulated Ca^{2+} in the cell in the ciliary response of *Paramecium* was first pointed out by Kamada (1938, 1940). Jahn (1962) reanalyzed the data of Kamada and Kinosita (1940) on the ciliary reversal in terms of the Gibbs-Donnan ratio, $[K^+]/\sqrt{[Ca^{2+}]}$, where $[K^+]$ and $[Ca^{2+}]$ are concentrations of K^+ and Ca^{2+} in the external medium. The reversal occurred when the Gibbs-Donnan ratio sufficiently increased, and the maximal duration of reversal was found at the same value of the Gibbs-Donnan ratio, regardless of dilution of the medium.

As shown in the previous paper, cation channels from the ciliary membrane of *Tetrahymena* were incorporated into planar lipid bilayers (Oosawa and Sokabe, 1985). These channels were permeable to monovalent cations, where the sequence of permeability was in the order: $K^+ > Li^+ \geqq Na^+$ (Oosawa and Sokabe, 1985). We report here that these channels are permeable not only to monovalent cations but also to Ca^{2+}.

Calcium channels that are permeable not only to divalent cations but also to monovalent cations have been found in skeletal muscle (Almers and McCleskey, 1984), cardiac muscle (Hess et al., 1986; Hess and Tsien, 1984), snail neuron (Kostyuk et al., 1983), and transverse tubule (Coronado and Affolter, 1986). The nicotinic acetylcholine receptor is also permeable to monovalent and divalent cations (Dani and Eisenman, 1987). Several models of ion permeation of these channels have been proposed.

We propose here a new model for the permeation of monovalent and divalent cations through the channel, where either two monovalent cations or one divalent cation are assumed to bind to the binding sites in the pore. This assumption is based on the fact that the Gibbs-Donnan equilibrium can explain the single-channel conductance in mixed solutions of K^+ and Ca^{2+}. This model predicts single-channel conductance in mixed solutions of monovalent and divalent cations and the dependence of swimming behavior on the Gibbs-Donnan ratio $[K^+]/\sqrt{[Ca^{2+}]}$.

MATERIALS AND METHODS

Ciliary membrane vesicles were prepared from *Tetrahymena thermophila* (strain BII from a stock kindly provided by Dr. M. Takahashi, University of Tsukuba) as described before (Oosawa and Sokabe, 1985) by the method of Adoutte et al. (1980) except that 10 mM Hepes-NaOH pH 7.4 was used instead of 10 mM Tris pH 8.0 after deciliation. Planar bilayers composed of 14.7 mg lecithin (type IIs) from Sigma Chemical Co., St. Louis, MO, in 1 ml n-decane were formed by the painting method of Mueller and Rudin (1969). The membrane conductance was measured by a current to voltage converting circuit under voltage clamp conditions. The amplifier output was recorded on a chart recorder and an FM tape recorder and later analyzed by hand. Experiments were done at room temperature, 18–24 °C. The *cis* compartment is defined as the compartment to which vesicles are added. The opposite compartment is defined as the *trans* compartment. The value of the holding potential refers to the potential of the *cis* compartment with respect to the *trans* compartment which was held at virtual ground.

RESULTS

The channel from *Tetrahymena* cilia that was incorporated into planar lipid bilayers was permeable to K^+, Na^+,

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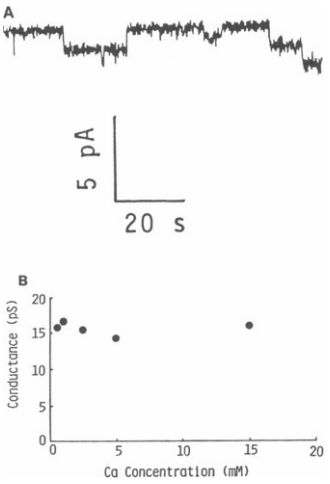

FIGURE 1 *A*. Single-channel current fluctuations in 50 mM Ca-gluconate at +50 mV. A downward deflection corresponds to a channel opening event. *B*. Single-channel conductance at various concentrations of Ca-gluconate. Each point represents the average of single-channel conductances found at +25 mV. n = 10–76. At 50 mM Ca^{2+} the single-channel conductance was 20.5 pS (n = 26, data not in the figure).

and Li^+ (Oosawa and Sokabe, 1985). The single-channel conductance at high concentration of K^+ was ~350 pS. This channel also was permeable to divalent cations such as Ca^{2+} and Ba^{2+} (Fig. 1 *A*). The single-channel conductance for Ca^{2+} was almost independent of the concentration of Ca^{2+}. It was between 14.3 and 20.5 pS in a concentration range of Ca-gluconate from 0.5 to 50 mM, suggesting that the binding of Ca^{2+} to the site in the channel was very strong and the channel conductance was already saturated at 0.5 mM (Fig. 1 *B*).

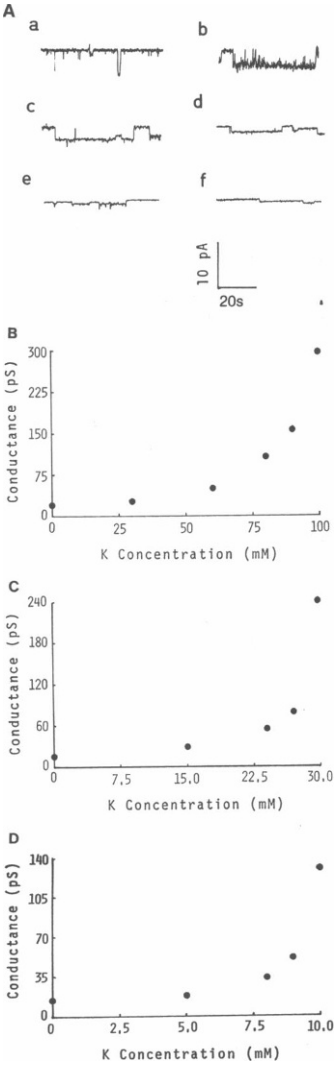

FIGURE 2 *A*. Single-channel records in symmetric solutions at +25 mV. (*a*) 100 mM K^+. (*b*) 90 mM K^+, 5 mM Ca^{2+}. (*c*) 80 mM K^+, 10 mM Ca^{2+}. (*d*) 60 mM K^+, 20 mM Ca^{2+}. (*e*) 30 mM K^+, 35 mM Ca^{2+}. (*f*) 50 mM Ca^{2+}. A downward deflection corresponds to a channel opening event. *B, C, D*. Single-channel conductance in symmetric mixed solutions (K-gluconate and Ca-gluconate). $[K^+] + 2[Ca^{2+}]$ = 100 mM(*B*), $[K^+] + 2[Ca^{2+}]$ = 30 mM(*C*), $[K^+] + 2[Ca^{2+}]$ = 10 mM(*D*). The data are plotted as conductance versus concentration of K^+. Each point represents the average of the single-channel conductances found at +25 mV.

In the experiments with mixed solutions where the concentrations of K^+ and Ca^{2+} were varied, keeping $[K^+] + 2[Ca^{2+}]$ constant, single-channel conductances changed, as shown in Fig. 2. The measurements were made by applying +25 mV with the same solution in the *cis* and *trans* compartment. With the increase of the ratio of $[K^+]$ to $[Ca^{2+}]$, the conductance increased very much. This must be due to the bound Ca^{2+} in the channel being replaced with K^+.

In these experiments we found that the single-channel conductance was similar in similar values of the Gibbs-Donnan ratio ($[K^+]/\sqrt{[Ca^{2+}]}$) (Table I). Therefore, we postulate a Gibbs-Donnan equilibrium between the binding sites and outside ions (Ca^{2+} and K^+). The number of the binding sites in the channel is two. Either one calcium ion or two potassium ions are bound to these sites in our experimental condition ($[K^+]$: 5–90 mM and $[Ca^{2+}]$; 0.5–35 mM), as described by the binding equilibrium;

$$B_2Ca + 2K^+ \rightleftharpoons B_2K_2 + Ca^{2+} \quad (1)$$

$$\frac{[B_2K_2][Ca^{2+}]}{[B_2Ca][K^+]^2} = k, \quad (2)$$

where B represents the binding site of the channel. As far as Ca ions above 0.5 mM are present in the medium, binding sites cannot be free. Two binding sites are assumed to bind two potassium ions or one calcium ion.

$$[B_2Ca] + [B_2K_2] = 1 \quad (3)$$

If $[B_2K_2]$ equals α then $[B_2\,Ca]$ equals $1 - \alpha$. The channel conductance is given by the sum of conductances of K^+ and Ca^{2+} which permeate the channel through the bound state.

TABLE I
SINGLE-CHANNEL CONDUCTANCES IN SOLUTIONS OF K^+ AND Ca^{2+}

$[K^+]$	$[Ca^{2+}]$	$\gamma \pm$ SE (n)	$[K^+]/\sqrt{[Ca^{2+}]}$
mM	*mM*	*pS*	$\sqrt{M}$
90	5	154 ± 9.9 (10)	1.27
80	10	106 ± 5.0 (26)	0.8
60	20	49.8 ± 1.6 (10)	0.42*
30	35	27.2 ± 2.5 (18)	0.16‡
27	1.5	78.4 ± 2.8 (8)	0.7
24	3	54.5 ± 1.5 (19)	0.44*
15	7.5	29.6 ± 1.7 (12)	0.17‡
9	0.5	51.1 ± 2.2 (10)	0.4*
8	1	34.0 ± 1.5 (11)	0.25
5	2.5	18.8 ± 0.8 (21)	0.1

Same data in Fig. 2, *B–D* were used. Similar Gibbs-Donnan ratios ($[K^+]/\sqrt{[Ca^{2+}]}$) lead to similar single-channel conductances (see * or ‡).

The exchange of bound K^+ and Ca^{2+} is assumed to be much faster than the open-close fluctuation of the channel. Then,

$$\gamma_{total} = \alpha\gamma_K^{max} + (1 - \alpha)\gamma_{Ca}^{max} \quad (4)$$

$$\alpha = \frac{\gamma_{total} - \gamma_{Ca}^{max}}{\gamma_K^{max} - \gamma_{Ca}^{max}}, \quad (5)$$

where γ_K^{max} and γ_{Ca}^{max} are maximum channel conductances of K^+ and Ca^{2+}, respectively, and γ_{total} is the total channel conductance. From Eq. 2,

$$\frac{\sqrt{\alpha}}{\sqrt{1 - \alpha}} = \sqrt{k}\frac{[K^+]}{\sqrt{[Ca^{2+}]}}. \quad (6)$$

From Eqs. 5 and 6

$$\frac{\sqrt{\gamma_{total} - \gamma_{Ca}^{max}}}{\sqrt{\gamma_K^{max} - \gamma_{total}}} = \sqrt{k}\frac{[K^+]}{\sqrt{[Ca^{2+}]}}. \quad (7)$$

Thus, γ_{total} is determined by the Gibbs-Donnan ratio $[K^+]/\sqrt{[Ca^{2+}]}$. By putting γ_K^{max} and γ_{Ca}^{max} to be 350 and 20 pS, respectively, the left side of Eq. 7 was calculated from the observed γ_{total} in the condition of various ratios, $[K^+]/\sqrt{[Ca^{2+}]}$. The data of the conductance in the Ca^{2+}-free solution were not included in the analysis, because in the absence of Ca^{2+}, the conductance of K^+ was not saturated, and the Gibbs-Donnan equilibrium was not satisfied. As shown in Fig. 3, experimental data gave linear relationship in good agreement with Eq. 7. This means that the Gibbs-Donnan equilibrium is satisfied between cations, K^+, Ca^{2+}, and the binding sites of the channel in our experimental condition ($[K^+]$: 5–90 mM and $[Ca^{2+}]$; 0.5–35 mM). The equilibrium constant (k) was calculated from the slope of the straight line of Fig. 3. It was $(0.698)^2 = 0.487\ M^{-1}$. With this k, all data in mixed solutions are in good agreement with Eq. 7.

DISCUSSION

We found that the cation channel of *Tetrahymena* cilia is permeable to monovalent and divalent cations, and the Gibbs-Donnan theory is useful to analyze the single-channel conductances in the K^+ and Ca^{2+} mixed solutions. Until now the Gibbs-Donnan equilibrium was usually applied to the binding of the cations to the membrane (Naitoh and Yasumasu, 1967) which would change the effective membrane potential. However, our analysis indicates that the channel conductance itself is controlled by the Gibbs-Donnan ratio.

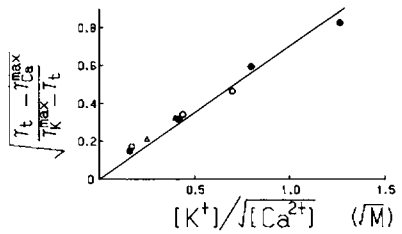

FIGURE 3 Application of the Gibbs-Donnan equilibrium to the data. Ordinate represents the values on the left side of Eq. 7. Abscissa represents the Gibbs-Donnan ratio. The straight line passes through the origin of the graph. $[K^+] + 2[Ca^{2+}] = 100$ mM (*filled circle*), 30 mM (*open circle*), 10 mM (*open triangle*).

Some calcium channels are permeable to monovalent and divalent cations. Almers and McCleskey (1984) reported that a skeletal muscle calcium channel is permeable to monovalent and divalent cations, and proposed a model of the channel as a single-file multi-ion channel. Similar behavior has been reported of a cardiac muscle calcium channel (Hess and Tsien, 1984) and a transverse tubule calcium channel (Coronado and Affolter, 1986). Kostyuk et al. (1983) proposed that a snail neuron calcium channel had a single intrachannel binding site and monovalent ion currents were blocked by an external site for calcium ion. Dani and Eisenman (1987) proposed that nicotinic acetylcholine receptors had wide multiply-occupied vestibules that served as transition zones to the singly-occupied narrow region. They reported single-channel currents of acetylcholine receptor in mixed solution of monovalent and divalent cations with some analysis of their data, and the theory was presented in depth by Dani (1986). Almers and McCleskey (1984) and Hess and Tsien (1984) reported that membrane currents in Ba^{2+}/Ca^{2+} mixture solutions (both divalent cations) showed anomalous mole-fraction behaviors, and they proposed a multi-ion channel model. Fox and Ciani (1985) reported that a sarcoplasmic reticulum cation channel showed anomalous mole-fraction behaviors in K^+/Tl^+ mixture solutions (both monovalent cations) and they proposed the existence of two external modulatory sites on the channel, without implying double-occupancy in the permeation pathway. We have done a quantitative analysis of single-channel conductance in K^+/Ca^{2+} mixture solutions of the cation channel from *Tetrahymena* cilia because this channel is always faced with monovalent and divalent cations under physiological conditions.

We postulate that the channel has binding sites which are always saturated with two K^+ or one Ca^{2+}. This postulation is reasonable because the channel is saturated by 0.5 mM Ca^{2+} and under our experimental condition the solution always had more than 0.5 mM Ca^{2+}. The sites may have two negative charges, but we have no data about the structure of the sites. Our model can also be applied to other electrophysiological experiments with *Paramecium* and *Tetrahymena* because in those experiments the outer solutions usually contained several milimolar potassium and calcium ions (Naitoh and Yasumasu, 1967; Satow and Kung, 1979; Machemer and Ogura, 1979; Onimaru et al., 1980; Connolly and Kerkut, 1981), and the intracellular potassium concentration was 30 mM (Dunham and Child, 1961). Under these conditions our postulation that the binding sites of the channel are always saturated with two potassium ions or one calcium ion is reasonable. Our result may give a theoretical basis for application of the Gibbs-

Donnan theory to the analysis of the ciliary response of *Paramecium*.

Eq. 7 derived from the Gibbs-Donnan equilibrium may help to understand the single-channel conductances of cation channels, that are permeable to monovalent and divalent cations, in other biomembranes in mixed solutions of monovalent and divalent cations.

We thank Dr. Masahiro Sokabe for showing us the technique of vesicle fusion and Dr. Fumio Oosawa for helpful comments.

Received for publication 7 December 1987 and in final form 8 March 1988.

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