# Reconstitution of the voltage-gated $K^+$ channel KAT1 in planar lipid bilayers

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KAT1 channel is a $K^+$-selective ion channel cloned from higher plant *Arabidopsis thaliana* [1,2]. It is located in guard cells and belongs to voltage-gated *Shaker*-type channels comprising four subunits. KAT1 regulates stomatal movement as the inward-rectifying $K^+$ channels, which is activated by hyperpolarization. The electrophysiological properties of KAT1 in heterologous expression systems such as oocytes of *Xenopus laevis*, insect cells and yeast have been investigated by patch clamp method [3,4]. The channel properties including ion selectivity, voltage dependency and sensitivity against inhibitors were different among the KAT1 channels expressed in various systems [5,6].

The planar bilayer lipid membrane (BLM), in which isolated ion channels are reconstituted, has also been employed in order to analyze electrophysiological properties of ion channels and transporters. Compared with the patch clamp method, this method has several advantages as follows; (i) The effect of endogenous proteins and compounds in the expression systems can be minimized. (ii) The experimental conditions such as lipid components and ionic compositions are strictly defined. These advantages are important to investigate the effect of inhibitors, modulators and coexisting ions on the channel properties such as the magnitude of single-channel current and its dependence on the applied potential.

In the present work, the method for the reconstitution and single-channel recording of KAT1 in the planar BLM system was constructed in order to elucidate the detailed mechanisms for the ion transport facilitated by KAT1 channel.

The expression and purification of KAT1 channel were performed using the same procedures as previously described [7]. The channel protein was solubilized from the membrane fraction of the cultured cell. KAT1 was then reconstituted into preformed asolectin liposomes by freeze-thaw method [8].

The electrochemical cell used for single channel recordings was essentially the same as that used in our previous work [9]. Two unsymmetrical aqueous compartments, W1 and W2, were filled with 9 mL and 3 mL of buffer solutions (100 mM KCl and 20 mM Tris pH 7), respectively. They were separated by a 0.2-mm thick tetrafluoroethylene resin sheet. The BLMs were obtained as black lipid membranes by brushing a BLM-forming solution on the 1-mm diameter aperture created on the tetrafluoroethylene resin sheet. The BLM-forming solution was prepared by dissolving 10 mg of asolectin and 5 mg of Ch in 1 mL of *n*-decane. After the formation of the BLM in the electrochemical cell, 20-50 μL of the liposome solution containing KAT1 was added to W2 with constant stirring to promote the fusion of proteoliposomes with the BLM.

Single-channel currents between W1 and W2 across the BLM were recorded by applying the constant potential difference between W1 and W2, $E_{W1\text{-}W2}$, and measuring the current between W1 and W2, $I_{W1\text{-}W2}$.

The observed value of bilayer area were about $5 \times 10^{-3}$ $cm^2$ on average. These BLMs were stable for about 1.5 h and no conductance fluctuation was observed. After the fusion of the proteoliposomes with the BLM, the current fluctuation was observed between W1 and W2, as shown in Fig 1. Any current fluctuations were not caused by the addition of the detergent, lipid components and other chemicals. Therefore, it is assumed that the ion channel reconstituted in the BLM is responsible for the current fluctuation. Channel opening and closing events (O and C) were seen when $E_{W1\text{-}W2}$ was greater than 60 mV. Figure 1 shows the representative single-channel traces observed at $E_{W1\text{-}W2}$ ranging from 60 mV to 120 mV. The dashed line in each recording represents the closed state of the channel. At 120 mV, one or two channels were served ($O_1$ or $O_2$). In the potential region less than 60 mV, the current fluctuation caused by channel opening and closing events hid behind the background electric noise. Single-channel currents at $E_{W1\text{-}W2}$ ranging from 60 mV to 120 mV were proportional to $E_{W1\text{-}W2}$. The voltage-independent conductance of the reconstituted channel was ranging from 10 pS to 12.5 pS. These are close to the reported values [5,6].

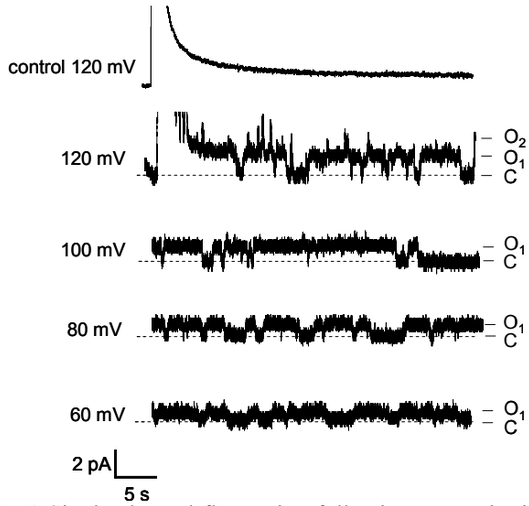


Fig. 1 Single-channel fluctuation following reconstitution of the channel into the BLM.

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