INTRODUCTION

The ion interactions with KcsA from *Streptomyces lividans* were studied by $^{87}\text{Rb}$ NMR. KcsA is a tetrameric potassium channel with 160 amino acids and its structure has been determined by x-ray crystallography. This channel has very high conductivity ($10^8$ ions per second). $\text{Rb}^+$ has a similar ionic radius (1.48Å) to that of $\text{K}^+$ (1.33Å) with similar conductivity through this KcsA channels. With a natural abundance of 27.8% and relative sensitivity of 0.17, $^{87}\text{Rb}$ is a good substitute for $\text{K}^+$ to study potassium channel with NMR. $^{87}\text{Rb}$ has a spin of 3/2 and its central transition will be studied here.

EXPERIMENTAL

KcsA was overexpressed in *E. Coli*, purified and reconstituted in DOPC/DOPG (4:1) liposomes with a protein/lipid molar ratio of 1:100. The proteoliposomes were equilibrated with an appropriate rubidium concentration (2, 5, 15, 30, or 60mM) and were pelleted by ultracentrifugation.

RESULTS AND DISCUSSION

Figure 1 shows the $^{87}\text{Rb}$ NMR spectra for the DOPC/DOPG liposomes with/without KcsA protein. Without KcsA, there is only one peak (Figure 1a, 0 ppm, linewidth 300 Hz) and the peak-width decreases with increasing rubidium concentration. This suggests that there is fast-exchange (> 10 Hz) between $\text{Rb}^+$ ions in bulk water and those interacting with lipid bilayers. When KcsA protein is incorporated into the bilayers, two peaks are observed (Figure 1b). The sharp peak (0 ppm, linewidth 150 Hz) has same peakwidth as $\text{Rb}^+$ in bulk solution and the broad peak (1.3 ppm, linewidth 700 Hz) appear to arise from the fast exchange between lipid-bound rubidium ions and KcsA-bound ions. The positions and peak widths of two peaks show no significant change at different rubidium concentrations.

Conclusions

The $^{87}\text{Rb}$ NMR study supported a two-step model for how ions enter the channel from bulk solution. The rubidium ions bind to the lipid bilayers first and then the lipid-bound rubidium ions transfer to the protein’s pore region of the channel. The exchange rate of the second step is fast (>> 300 Hz). The rate of the first step is greater than 10 Hz but slower than the second step. The binding constant of rubidium ions to KcsA was estimated to be 65 M$^{-1}$ when [Rb$^+$] = 2mM, by assuming a first order binding process. The two-step model suggests a novel way for Ca$^{2+}$ to inhibit the channel conductance by depleting the surrounding lipid of $\text{Rb}^+$ or $\text{K}^+$ ions.

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