SUPPLEMENTAL MATERIAL

for the manuscript by

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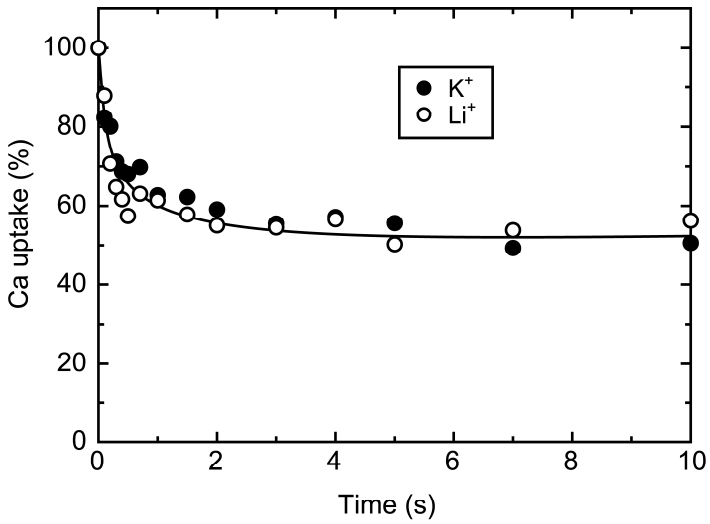
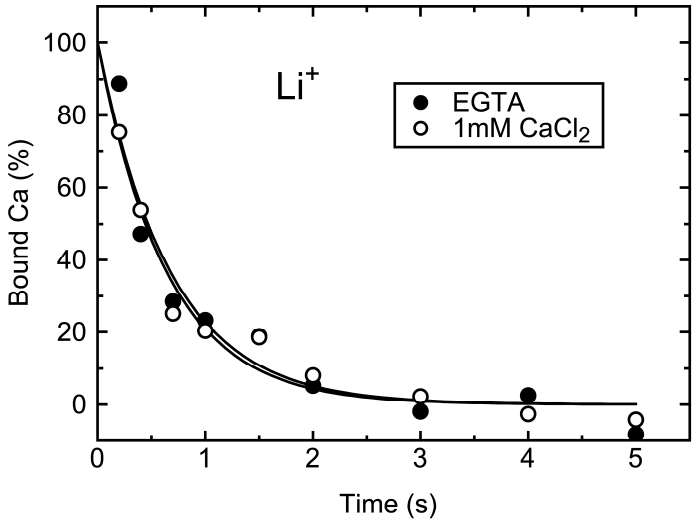
Ca^{2+} Release to Lumen from ADP-sensitive Phosphoenzyme *E*1PCa_2 without Bound K^+ of Sarcoplasmic Reticulum Ca^{2+}-ATPase



Supplemental Figure S1.
Labeling Site I by $^{45}Ca^{2+}$.
SR vesicles (20 μg/ml) were incubated with 10 μM $^{45}CaCl_2$ for ~10 min, then diluted by an equal volume of a solution containing 2 mM non-radioactive $CaCl_2$. After the subsequent incubation for the indicated time periods, Ca^{2+} uptake in a single turnover of *E*P was initiated by mixing with an equal volume of a solution containing 20 μM ATP and 2 mM EGTA. Immediately, the sample was spotted on the membrane filter and washed for ~10 s by the EGTA solution as in Fig. 4, and the amount of $^{45}Ca^{2+}$ uptake was determined. All the solutions contained 0.1 M KCl (*closed circles*) or LiCl (*open circles*).



Supplemental Figure S2.
Non-sequential $^{45}Ca^{2+}$ release from E1PCa$_2$ without K^+. SR vesicles (20 μg/ml) were phosphorylated for 30 s with 10 μM ATP in 10 μM $^{45}CaCl_2$, 3 μM A23187, and 0.1 M LiCl. Then an aliquot of the solution was spotted on the membrane and washed by a washer containing 0.1 M LiCl, 3 μM A23187, and non-radioactive 1 mM $CaCl_2$ (*open circles*) or 2 mM EGTA (*closed circles*) for the indicated time periods. The amounts of $^{45}Ca^{2+}$ specifically bound to the Ca^{2+}-ATPase were determined. *Solid lines* show the least squares fit to a single-exponential.

Note, if the Ca^{2+} release is sequential, the biphasic $^{45}Ca^{2+}$ release would take place when the first released $^{45}Ca^{2+}$ is exchanged by non-radioactive 1 mM Ca^{2+} (which is high enough for binding to the Ca^{2+} sites (Fig 6). We found the same single-exponential $^{45}Ca^{2+}$ release kinetics upon the addition of excess EGTA and that of 1 mM Ca^{2+}. The results show that the Ca^{2+} release from *E*1PCa$_2$ in the absence of K^+ is non-sequential, as previously observed in the presence of K^+ for the normal release process *E*1PCa$_2$ → *E*2PCa$_2$ → *E*2P + 2Ca^{2+} (58, 59).