AlF_4^- is a potent inhibitor of PMCA that promotes Ca^{2+} occlusion

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$AlCl_3$ forms a complex with F^- known as fluoroaluminate (AlF_4^-), with structure very similar to $H_2PO_4^-$. It is an inhibitor of P-type ATPases (Na, K- ATPase, SERCA and PMCA). In this work we investigated the inhibitory properties of AlF_4^- in the PMCA of pig erythrocyte ghosts, relating this inhibition with the occlusion of Ca^{2+}. PMCA (1 mg/mL ghost) was preincubated with AlF_4^- (mix of 1 mM NaF with different concentrations of $AlCl_3$) and 50 μM ADP at 37 ^{o}C. Afterward, membranes were washed and ATPase activity were assayed in media containing 30 μM Ca^{2+} or 300 μM EGTA. The difference of both ATPase activities was attributed to PMCA. AlF_4^- inhibited irreversibly PMCA in a time-dependent manner. The ions Mg^{2+} and Ca^{2+} seemed to be important to promote this inhibition. The profile of inhibition at Increasing concentrations of AlF_4^- (0.5, 2 and 10 μM) suggests a single site for inhibitor binding. ATP or AMP-PNP in the preincubation protected PMCA against inhibition, suggesting that AlF_4^- binds at ATP site of the enzyme. Moreover, 10 μM AlF_4^- promoted Ca^{2+} occlusion when incubated for 30 minutes at room temperature. These findings showed that PMCA is an enzyme that transports Ca^{2+} in a mechanism similar to proposed to SERCA, passing through an intermediary step in its catalytic cycle, in which Ca^{2+} is occluded.

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