

Inhibition of PMCA by AlF_4^-

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AlCl_3 forms a complex with F^- known as fluoroaluminate (AlF_4^-), with molecular structure very similar to H_2PO_4^- , which is an inhibitor of P-type ATPases (Na,K-ATPase, SERCA and PMCA). In this work we investigated the inhibitory properties of AlF_4^- on the phosphatase and ATPase activities of the PMCA of pig erythrocyte ghosts. Ghosts were preincubated with AlF_4^- (mix of 0.6 mM NaF with different concentrations of AlCl_3) and 50 μM ADP at 37°C. Afterward, membranes were washed with media containing deferoxamine and ATPase or phosphatase activities, attributed to the E2 form of the enzyme, assayed in media containing 30 μM Ca^{2+} or 300 μM EGTA. The difference of both activities was attributed to PMCA. AlF_4^- inhibited irreversibly PMCA in a time-dependent manner. The ion Mg^{2+} and calmodulin seemed to be important to promote this inhibition. The complex AlF_4^- reacted slowly ($T_{1/2} = 50\text{-}60$ min) and irreversible with the PMCA. The apparent affinity for the inhibitor was about 1 μM , independent of the activities were measured at 5 or 1000 mM ATP. The same K_i was observed for the inhibition of the phosphatase activity, suggesting a single site for tight binding of AlF_4^- at the ATP binding site. ATP or AMP-PNP in the preincubation protected PMCA against inhibition. These findings showed that PMCA is an enzyme that transports Ca^{2+} in a mechanism similar to proposed to SERCA and That AlF_4^- can be used as a tool for kinetic and structural studies of the PMCA.

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