

CHARACTERIZATION OF THE INHIBITION OF THE PMCA BY BeF_x

Carvalho, A.P., Oliveira, V.H., Scofano, H.M., & Mignaco, J.A.

Instituto de Bioquímica Médica – CCS/UFRJ, RJ, Brazil.

The Plasma Membrane Ca²⁺ pump (PMCA), fundamental for cell Ca²⁺ homeostasis, is a high affinity, low capacity, ATP dependent calcium transporter of the P-type ATPase family. It is reported that AlF_x, MgF_x and BeF_x inhibit P-type ATPases, possibly by interacting with their phosphate-binding site. In a previous communication we reported a very slow inhibition of PMCA by BeF_x at pH 7.4 (Carvalho et al. SBBq 2006). In this report we further characterize the action of BeF_x on the enzyme. Incubation of PMCA with 50μM BeF_x for >360 min partially inhibited ATP hydrolysis, in presence or absence of ADP. Time-dependence experiments showed that shifting the pre-incubation pH to 6,0 complete and irreversibly inhibited the hydrolytic activity of ghosts in <120 minutes, either in presence or absence of calcium. BeF_x complex also inhibited the hydrolysis of pNPP, developed by the E₂ conformation either in EGTA or in presence of Ca²⁺-CaM, possibly by binding at the phosphate site. These data suggest that the BeF_x complex can bind to either the E₂ or E₁Ca conformation of PMCA and drive it to an E_(?)(Ca?)BeF_x inactive complex that remains stable after addition of ATP or Ca⁺². Possibly BeF_x formed a “dead end” complex in the E₂BeF_x conformation as described for other P-type ATPases and further structural information should confirm this data.

Sponsors: CAPES, CNPq, FAPERJ.

mailto: honorato@bioqmed.ufrj.br

Keywords: PMCA, BeF_x, Calcium