FUNCTIONAL CONSEQUENCES OF ALTERATIONS TO W854 AND W855 LOCATED IN THE M7-M8 LOOP OF THE CA^{2+}-ATPASE OF SARCOPLASMIC RETICULUM

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Ca^{2+}-ATPase of sarcoplasmic reticulum (SR) is a membrane-bound enzyme that sustains active transport of Ca^{2+} from the cytosol to the lumen, coupled to utilization of ATP. Functional analysis of two tryptophan of Ca^{2+}-ATPase, located in the M7-M8 lumenal loop (W854 and W855), was performed changing tryptophans to either alanine (A) or phenylalanine (F). W854A and W855F mutations allow good expression, but produced strong inhibition of Ca^{2+}-ATPase activity. The main defect produced by these mutations is strong interference with enzyme phosphorylation by ATP in the presence of Ca^{2+}, and also by Pi in the absence of Ca^{2+}. The phosphoenzyme of mutants appeared unable to react with ADP, suggesting that ADP-insensitive E2P had accumulated at steady state. Mutants were analyzed with respect to their sensitivity to 1,3-dibromo-2,4,6-tris(methylisothiouronium)-benzene (Br_2-TITU) inactivation. Higher levels of phosphoenzyme formed from ATP and Ca^{2+} were accumulated for wild-type and W854 mutants in the presence than in the absence of Br_2-TITU. In contrast, W855 mutants lost Br_2-TITU inhibition. These results indicate that the inhibition of Ca^{2+}-ATPase by Br_2-TITU occurs through the interaction with W855 that is conserved in all Ca^{2+}-ATPases and Na^+,K^+-ATPases. Functional and structural analysis of the experimental data has pointed to two tryptophan residues playing a critical role in the catalytic cycle of the SR Ca^{2+}-ATPase.

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