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The promiscuous phosphomonoestearase activity of *Archaeoglobus fulgidus* CopA, a thermophilic Cu^+ transport ATPase

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Abstract

Membrane transport P-type ATPases display two characteristic enzymatic activities: a principal ATPase activity provides the driving force for ion transport across biological membranes, whereas a promiscuous secondary activity catalyzes the hydrolysis of phosphate monoesters. This last activity is usually denoted as the phosphatase activity of P-ATPases. In the present study we characterize the phosphatase activity of the Cu^+-transport ATPase from *Archaeglobus fulgidus* (*Af*-CopA) and compare it with the principal ATPase activity. Our results show that the phosphatase turnover number was 20 times higher than that corresponding to the ATPase activity, but it is compensated by a high value of K_m, producing a less efficient catalysis for pNPP. This secondary activity is enhanced by Mg^{2+} (essential activator) and phospholipids (non-essential activator), and inhibited by salts and Cu^+. Transition state analysis of the catalyzed and non catalyzed hydrolysis of pNPP indicates that *Af*-CopA enhances the reaction rates by a factor of 10^5 ($\Delta\Delta G^‡$ = 38 kJ/mol) mainly by reducing the enthalpy of activation ($\Delta\Delta H^‡$ = 30 kJ/mol) whereas the entropy of activation is less negative on the enzyme than in solution. For the ATPase activity the decrease in the enthalpic component of the barrier is higher ($\Delta\Delta H^‡$ = 39 kJ/mol) and the entropic component is small on both the enzyme and in solution. These results suggest that different mechanisms are involved in the transference of the phosphoryl group of p-nitrophenyl phosphate and ATP.

Abbreviations

Af-CopA, Cu(I)-transport ATPase from *Archaeglobus fulgidus*; DDM, n-dodecyl β-D-maltoside; DTT, dithiothreitol; HAD, haloacid dehalogenase; MOPS, 3-(N-morpholino)propanesulfonic acid; PC, phosphatidylcholine; PE, phosphatidylethanolamine; Pi, inorganic phosphate; X_{PL} phospholipid mole fraction in the micellar phase; PMCA, plasma membrane Ca^{2+}-ATPase;