Thermodynamics of ATP binding to the Na^+,K^+-ATPase

A.M. Pilotelle,[1] F. Cornelius,[2] H.-J. Apell,[3] J.M. Matthews[4] and R.J. Clarke,[1] [1]School of Chemistry, University of Sydney, NSW 2006, Australia, [2]Department of Biophysics, University of Aarhus, DK-8000 Aarhus C, Denmark, [3]Department of Biology, University of Constance, D-78457 Constance, Germany and [4]School of Molecular and Microbial Biosciences, University of Sydney, NSW 2006, Australia.

The Na^+,K^+-ATPase is a P-type ATPase, found in all animal cells. The enzyme uses the free energy from the hydrolysis of ATP for transporting Na^+ and K^+ ions across the cell membrane. Previous work using stopped-flow fluorimetry suggests that the enzyme exists as a dimer with two gears of pumping depending on the number of ATP molecules bound (Clarke & Kane, in press). To test this hypothesis isothermal titration calorimetric (ITC) experiments have been performed to see if evidence for two ATP binding equilibria could be detected. Initial experiments showed heat signals due to a very slow ATP hydrolysis in addition to ATP binding. These signals disappeared, however, on adding the specific Na^+,K^+-ATPase inhibitor, ouabain, which blocks the enzyme in a phosphorylated state. The slow ATP hydrolysis must, therefore, be due to Na^+,K^+-ATPase activity stimulated by small amounts of divalent metal ions, which act as ATP cofactors for hydrolysis. The slow ATP hydrolysis could also be inhibited by the inclusion in the buffer of 10 mM CDTA, a strong Mg^{2+} chelator. Under these conditions any heat signals measured could be confidently attributed to ATP binding alone. ATP binding was also investigated by measuring the fluorescence anisotropy of the fluorescent ATP derivative, BODIPY FL ATP (Molecular Probes), over a range of concentrations at constant protein concentration. When the probe binds to Na^+,K^+-ATPase a significant increase in its fluorescence anisotropy occurs due to the reduced rotational mobility of the fluorophore. Analysis of both the ITC and fluorescence anisotropy data enabled ATP dissociation constants to be determined and a critical discussion of the dimer Na^+,K^+-ATPase hypothesis.

Clarke RJ & Kane DJ. (2007) *Biophysical Journal*, in press.