Effects of Metal Ions on the Conformational Equilibria of the Na^+/K^+- and H^+/K^+- ATPases

D. Diaz[a], Flemming Cornelius[b] and Ronald J. Clarke[a,c]

[a]*School of Chemistry, University of Sydney, Sydney, NSW 2006, Australia;*

[b]*Department of Biomedicine, University of Aarhus, Aarhus, Denmark;*

[c]*The University of Sydney Nano Institute, Sydney, NSW 2006, Australia*

The Na^+,/K^+- and H^+/K^+-ATPases are closely related ion pumps. The Na^+/K^+-ATPase is responsible for maintaining the electrochemical potential gradients for Na^+ and K^+ across the plasma membrane of all animal cells, which are crucial for numerous physiological functions. The H^+/K^+-ATPase is responsible for stomach acidification. Both enzymes exist in two main conformations, E1 and E2. The distribution of the enzymes between these two states determines their relative affinities for their substrate ions. Recent results [1] showed that the distribution of the Na^+/K^+-ATPase between the E1 and E2 states depends on the ionic strength of the solution. It was suggested that this is due to ionic strength screening of an electrostatic interaction stabilizing the E2 state relative to the E1. Because prior experiments have shown that the lysine-rich N-terminus of the Na^+/K^+-ATPase undergoes significant movement during the E2-E1 transition and it is known that the surrounding membrane contains a high content of negatively charged lipids, e.g. phosphatidylserine (PS), it was hypothesized that the electrostatic interaction could be between the N-terminus and the surrounding lipid membrane [1].

In this study we investigated the effect of mono-, di- and trivalent metal ions on the E2-E1 distribution of both the Na^+,/K^+-ATPase and H^+/K^+-ATPase. The E2-E1 distribution was quantified via a ratiometric fluorescence method utilising the probe eosin. Eosin binds to the ATP binding site of both proteins, but its binding affinity is dependent on the enzyme conformation. Hence the proportion of enzyme-bound and free eosin changes when the enzyme undergoes its E2-E1 transition. This is reflected in both a change in fluorescence intensity and a shift in the maximum excitation wavelength. The results obtained showed that the conformational equilibria of the Na^+,/K^+- and H^+/K^+-ATPases are very sensitive to the presence of di- and trivalent metal ions. It is proposed that this could be due to specific binding of these ions to the negatively-charged headgroup of PS in the membrane around the proteins, thus causing release of the N-terminus from the membrane and stabilisation of the enzymes in the E1 conformation. Potentially such a mechanism could allow crosstalk between the Na^+,K^+-ATPase and the sarcoplasmic reticulum Ca^{2+}-ATPase in muscle cells via the cytoplasmic Ca^{2+} concentration and hence could play a role in the regulation of muscle contraction and relaxation.

[1] Jiang et al, *Biophys J* **112**, 288-299 (2017).