## APPARENT SUBSTRATE INHIBITION OF $Na^+$ $K^+$ ATPase IN MICROSOMAL PREPARATIONS FROM CRAB (*CARCINUS MAENAS*) GILL

Torstein Hølleland[1], Janet Wrestler[2] and David Towle[3]
[1]Zoological Laboratory, University of Bergen, 5007 Bergen, Norway
[2]University of Richmond, Richmond, VA 23173
[3]Department of Biology, Lake Forest College, Lake Forest, IL 60045

Determination of specific $Na^+$ $K^+$ ATPase activity is an important tool in describing ion transport mechanisms in osmoregulating animals (e.g., Hølleland, Shetlar, McDonald, Alexander and Towle, Bull. MDIBL 27: 63-65, 1987-1988). One way to quantitate the ATPase activity is by analyzing the inorganic phosphate production of ATP hydrolysis (Towle, Palmer and Harris, J. Exp. Zool. 196: 315-322, 1976). This abstract will present data showing apparent substrate inhibition of the $Na^+$ $K^+$ ATPase in microsomal gill preparations of the green shore crab (*Carcinus maenas*).

The crabs were collected and treated as described by Hølleland *et al.* (this volume). A microsomal preparation of gill epithelium was obtained as earlier described by Towle and Hølleland (Am. J. Physiol. 252: R479-R489, 1987) and Hølleland *et al.* (1987-88). Protein content of the final enzyme preparation (Bradford, Anal. Biochem. 72: 248-254, 1976) was between 1.5 and 2.5 mg/ml. Ten ul of this was used in 2 ml total assay volume. The ATP used was from Sigma (catalog number A5394, lots 76F-7040 and 116F-7080).

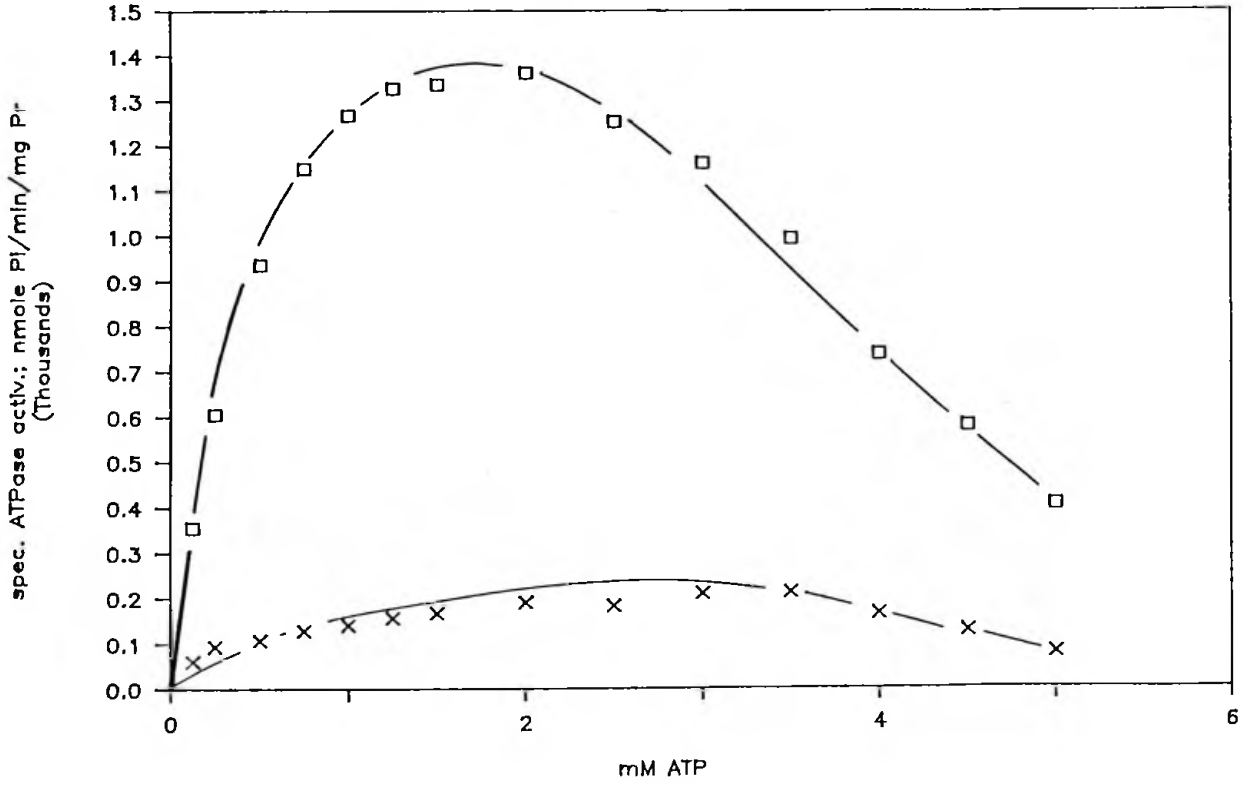


Figure 1. Specific ATPase activities ($Na^+$ $K^+$ ATPase as squares and $Mg^{++}$ as "X"es), given as nanomoles inorganic phosphate produced per minute per mg protein, are plotted against the concentration of ATP.

According to Sigma's specifications, this ATP has a low calcium content and is virtually free of vanadate, a strong $Na^+$ $K^+$ ATPase inhibitor (e.g., Hølleland *et al.*, 1987-88). Experiments were done at different ATP concentrations ranging from 0.1 to 5 mM. The $Mg^{++}$ concentration was constant in all assays (5 mM).

The maximum $Na^+$ $K^+$ ATPase activity was normally found between 1 and 2 mM ATP. For the ouabain-insensitive ATPase (the Mg ATPase) the maximum activity was found between 2 and 4 mM ATP (Fig. 1). Results from a test with two different lots of ATP showed that 5mM ATP gave less activity than 0.25 mM ATP (215 vs. 315 nmole Pi/min/mg protein). Calculation of kinetic constants using an Eadie-Hofstee plot showed no significant difference between the two lots of ATP ($K_m$ = 0.6 mM ATP). The marked inhibition of activity by ATP concentrations above 2 mM may result from true substrate inhibition or may reflect the presence of a contaminating inhibitor in the ATP. Preliminary results with ATP samples from other commercial sources, which produce little or no inhibition at high concentrations, supports the latter conclusion. Caution must be used therefore in interpreting kinetic data using Sigma's vanadate-free ATP.

Supported by NSF grant DCB-8711427 to DT.