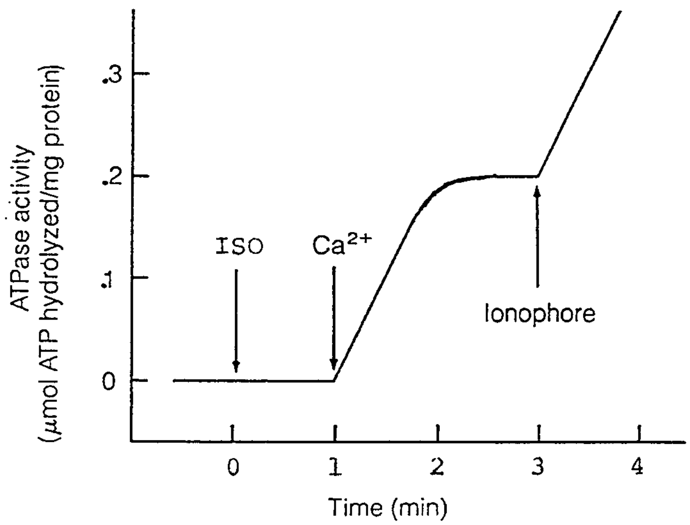
4. (32pts) In addition to the Na^+ and K^+ pump, the plasma membranes of many animal cells contain a Ca^+ pump that helps maintain cytoplasmic levels of Ca^+ (100 nM) four orders of magnitude *lower* than that present in the extracellular environment (1 mM). It's possible to study this pump using *inside-out* vesicles (ISO microsomes) that have been prepared from plasma membranes of erythrocyte ghosts or the homogenates of other cell preparations.

In the following experiment, an aliquot of ISO microsomes containing 2.0 mg protein was suspended in 1 ml (*final vol*) of a test medium containing 4 mM Mg^{2+}-ATP and 0.12 M KCl (at pH 7.4), and the hydrolysis of ATP was measured over time. After 1 min, 2.0 µMol of Ca^{2+} was added to the reaction mixture; 2 min later a Ca^{2+} ionophore (A23187) was added to the mixture. *Non of the additions changed the final volume of the reaction mixture.* The additions occurred at the times indicated by arrows.



Consider these data and answer **all** the following questions. **All** calculations must be shown to receive full credit, your reasoning must be explicit, and your explanations detailed.

A. (4pts) Why must ISO (in contrast to right-side-out) microsomes be used to study this particular calcium pump?

B. (4 pts) What is the *specific activity* of the pump ATPase, expressed in micromoles of ATP hydrolyzed per mg protein per min?

C. (7 pts) Why does ATP hydrolysis increase rapidly and then reach a plateau, following the addition of Ca^{2+}?

D. (6 pts) How does the addition of A23187 stimulate ATPase activity? Would you expect a second plateau? Why or why not?

E. (5 pts) Propose a test of your hypothesis for either question C. or D. and indicate clearly what the results would show.

F. (6 pts) How could you determine which membrane protein is the Ca^{2+} pump and estimate its molecular weight? (Note: some methods are better than others, involving fewer assumptions.)