REGULATION OF ORGANELLE ACIDITY

Introduction

Intracellular compartments are largely defined by their lumenal pH. Regulation of organelle acidity is a vital aspect of cellular homeostasis.

This lesson will show how to:

- Import predefined functions
- Import and fit experimental data

Additionally, we will explore in greater detail the mechanisms of ion transport.

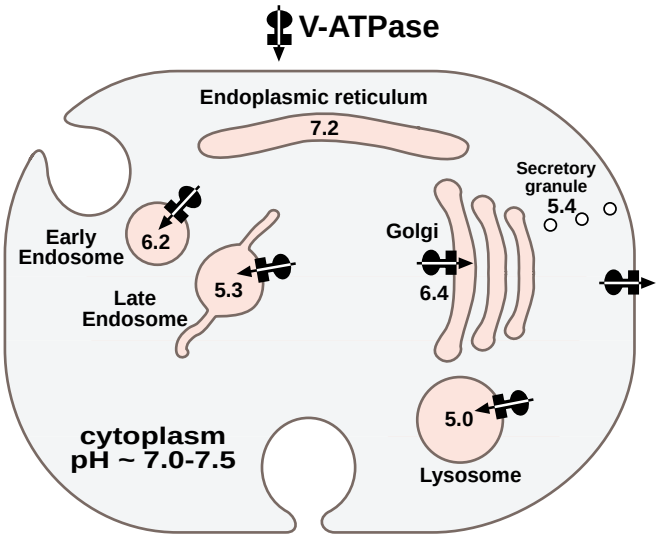

Figure 1. Typical organelle pH values.

A two compartment model

Biological Background

Acidity must be tightly regulated to sustain life. Blood pH is typically 7.4 pH units; when this value falls to just 7.2 severe acidosis ensues, and massive system failure follows and, if not treated , will result in death. Treatment involves the addition of buffer solutions to the blood. All cellular compartments maintain a distinctive pH that is essential to their function (see Figure 1); For example, lysosomes have pH ~ 5.0, in order to degrade harmful substances. The acidification of endosomes as they mature from early to late stages is required for the dissociation of receptors and ligands so that receptors can be recycled to the cell surface. A comprehensive understanding of how cellular organelles maintain their pH does not exist. As we will see, modeling can be a useful tool for interpreting experimental data.

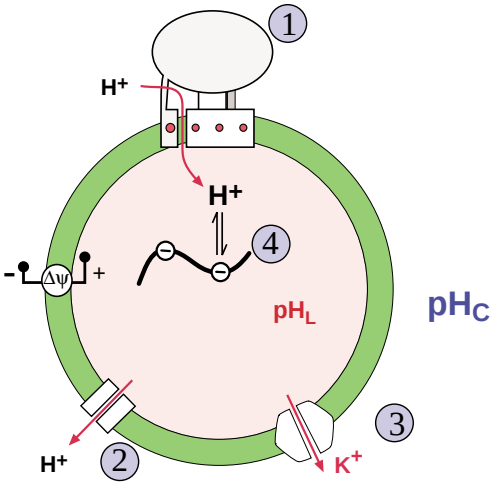

Figure 2. Key pH regulatory elements.
1 = V-ATPases, 2= proton leaks, 3 = K^+ leaks, 4 = lumenal buffering.

The principle components of organeller pH regulation are listed in Figure 2. It is believed that the competition between the proton pumping V-ATPase, **(**item #1**)**, and channel mediated proton leaking, (item #2), determines the steady state pH of many organelles. In crudest terms, this is like trying to file up a swimming pool with a bunch of holes in it. The pool will fill until the input hose is overpowered by the leaks. The case of ion transport is complicated by other factors. The lumen of the organelle buffers many of the protons that are pumped from the cytoplasm,(item #4). Additionally, as positive protons move across the organelle membrane a membrane potential builds up. This membrane potential is offset by the counter-movement of ions like potassium, (item #3). All of these effects can be combined into a coherent model.

Mathematical Description

THE V-ATPASE PROTON PUMP ACIDIFIES ORGANELLES

The hydrolysis of ATP provides the energy for pumping protons against their concentration gradient. For our purposes, we require the average pumping rate of a single V-ATPase as a function of membrane potential and pH gradient, J(ΔpH, ΔΨ). This function is defined numerically in the file **VATPASE**; it has been computed from another more complicated model.

THE PASSIVE LEAK OF IONS DEPENDS UPON CONCENTRATION AND MEMBRANE POTENTIAL

Intact bilayers are somewhat permeable to protons, but impermeable to other ions. Ion-specific channels allow an organelle to equilibrate specific ions between the lumen and cytoplasm. Movement of these ions through the channel is driven by the transmembrane concentration difference and the membrane potential. The simplest model for the diffusion flux of ions in the presence of a membrane potential can be described by

$$J_{H\ leak} = P_H \cdot S \cdot \frac{U \cdot \left([H^+]_L - [H^+]_C \cdot e^{-U}\right)}{1 - e^{-U}}$$
$$J_{K\ leak} = P_K \cdot S \cdot \frac{U \cdot \left([K^+]_L - [K^+]_C \cdot e^{-U}\right)}{1 - e^{-U}} \qquad (1)$$

where P is the permeability of the membrane to each ion, S is the surface area of the compartment, C refers to cytoplasmic concentrations, L refers to lumenal concentrations, and U is the reduced membrane potential, U = ΨF/(RT). F is Faraday's constant, R is the gas constant, and T is absolute temperature. The value of F/(RT) at room temperature is given in Table 2.

PROTONS BECOME BUFFERED AFTER THEY CROSS THE MEMBRANE

Cellular spaces are sponges for protons and other ions. Proteins and molecules are constantly binding and releasing ions from solution. The buffering capacity, β (units: [mol/pH]), measures the ability of the lumenal matrix is to bind protons. When protons cross the lipid bilayer a certain fraction of them are immediately bound and do not contribute to the pH. The change in proton concentration of the lumen and the change in pH are given by:

$$\Delta[H^+] = -\beta \cdot \Delta pH \tag{2}$$

In general, spaces have different buffering capacities at different pH values, but we will assume that the buffering power is a fixed constant. For measured values see Table 1.

ACCUMULATED CHARGE AS A MODEL FOR MEMBRANE POTENTIAL

The membrane potential affects the flow of ions across lipid membranes and biases the distributions of those ions at steady state. Electroneutrality requires every small volume be electrically neutral. The membrane potential arises from the microscopic deviation from electroneutrality at a lipid boundary. We use an explicit form for the membrane potential *across the bilayer* in terms of the excess charge inside the organelle. This treatment is very similar to the treatment of the membrane potential in the axon models. We assume that the net charge localizes to the lumenal leaflet, so that we can treat the membrane as a parallel plate capacitor. The potential drop across the bilayer is then written as:

$$\Delta\psi = \frac{F}{C_0} \cdot \frac{V}{A} \cdot \left(\underbrace{[K^+]_L}_{1} + \underbrace{\beta \cdot \left(pH_C - pH_L \right)}_{2} - \underbrace{B}_{3} \right) \tag{3}$$

where A is the surface area of the membrane, C_0 is the capacitance per unit area of the membrane ($C_0 \cdot A$ is the total capacitance of the membrane), V is the volume of the organelle, and the numbered terms giving the concentrations of charged particles are:

1. Total concentration of potassium ions.
2. Total amount of buffered and free protons in the lumen. β is the buffering capacity. We assume that protons do not contribute to the membrane potential when the lumenal pH is equal to the cytoplasmic pH.
3. Molar concentration of all impermeant charges. This term primarily represents fixed negative protein charges trapped in the lumen.

Despite the complexity of this system, it is only a two-tank model. In the next section we will construct this model in Berkeley Madonna™.

Assembling the model

Pure proton and potassium leak

Using the equations window, we begin by writing down differential equations for H and K, the lumenal proton and potassium concentrations, respectively. At this point, only include the passive leak for each time dependent variable (see equation (1)). In general, reservoirs should represent numbers of things. Let H and K be moles/liter (molarity). (Always check the units of terms!) Specifically, we use liters and centimeters (1000 cm^3 = 1 liter). Use the function pH = -log10(H) to represent the pH.

TECHNICAL NOTE. Often we encounter equations such as y = x/x that we want to evaluate when the denominator is equal to zero. In this case, the answer is 1 but if we ask the computer to evaluate this it will return a divide by zero error. Computers do not take limits easily. Equation (1) exhibits this problem when the membrane potential is equal to zero. The easiest way to handle this problem is to rewrite the equation in an equivalent form at the troubled areas. For equation 1, we use the form:

$$J_{H\,leak} = \begin{cases} P_H \cdot S \cdot \dfrac{U \cdot \left([H^+]_L - [H^+]_C \cdot e^{-U}\right)}{1 - e^{-U}} & for \ -.01 > U > .01 \\ P_H \cdot S \cdot \dfrac{\left([H^+]_L - [H^+]_C \cdot e^{-U}\right)}{1 - \dfrac{U}{2} + \dfrac{U^2}{6}} & for \ -.01 < U < .01 \end{cases} \quad (4)$$

Use the **IF** statement in Berkeley Madonna™ to implement this form of the leak term for the protons and the potassium.

Use the numbers for the Golgi from Table 1 and let, Ψ (Psi), equal zero to answer the following questions.

1. Start off with the lumenal pH = 5.4 and watch the proton concentration decrease until it is the same as the cytoplasmic value. It might be easiest to determine this by actually looking at the pH's. What is the time constant for the proton movement (use the plot of concentration, not the pH)?
2. Start off with the lumenal K^+ concentration at 20 mM. What is the time constant for this movement? The ratio of time constants is related to the ratio of permeabilities. When the model

reaches steady state, if the lumenal and cytoplasmic quantities are not equal then the channels are not being treated properly.

Accounting for proton buffering

Use $\Delta pH = -1/\beta \cdot \Delta[H^+]$ to rewrite the differential equation for the proton concentration. Now make the proton concentration a function just as we made pH a function above.

3. Compare the time constants for the lumenal proton concentration when the buffering is 0.01, 0.02, and 0.04 M. Notice that the proton movement is extremely slow now. Additionally, the change in pH is proportional to the proton concentration not the pH. Therefore, the solution is not an exponential and the time constant depends upon the initial values.

Couple in the membrane potential

So far the leak terms should be well behaved. When we add in the membrane potential, which couples the ion flows, we might find that our equations are not quite right. Expect problems here. Represent the membrane potential as in equation (3). Let the Donnen particle concentration, B = 0.1 M. We must enforce electroneutrality at the start of the simulation. We do this with the initial potassium concentration. Set INIT K = $B-\beta\cdot(pH\_c-pH)$. Explore the model and once you are convinced that it is giving reasonable results continue.

Add in proton pumping

Select Data Sets and import the function VATPASE as a 2-D matrix. We can set the pump rate equal to this function by defining it like this:

$$J_{pump} = N\_pump * \#VATPASE(psi, pH) \quad (5)$$

This function returns the number of protons per second per pump. What factors must this function be multiplied by in order to be used in the differential equation for pH? Write the correct form of the equation into your model. You must define a new parameter, N_pumps = number of pumps on the organelle membrane. Once the entire model is complete move on to the case studies.

NOTE: Predefined numerical functions are only defined over a certain domain. If during a simulation your variables extend beyond this domain the predefined function will not give the correct results. VATPASE is defined for $4.0 < pH < 7.6$ and $-80 \text{ mV} < \Psi < 260 \text{ mV}$.

Case 1. Endosomal acidification

Endosomes have been extracted from cells and are bathing in a 7.4 pH solution with 140 mM K^+. The V-ATPase proton pumps are not working because the solution lacks ATP. The pH of the endosome is measured using pH sensitive dyes. At time=37 seconds, ATP is reintroduced to the bathing solution and the endosomes begins to acidify from pH 7.4. Load the file MVB_74 into your model (this is the acidification data).

4. Using the endosome (MVB) parameters from Table 1 determine the number of V-ATPase pumps and the proton permeability by fitting the model pH to the experimental data. Using the fact that the endosomal pH = 7.4 in the absence of any proton pumping, determine the concentration of donnan particles.

Case 2. Membrane leakiness

We want to determine the proton permeability of the Golgi and secretory granule. We have provided you with two data sets each containing five experiments:

Data Set #1 - [SG_1; SG_2; SG_3; SG_4; SG_5]

Data Set #2 - [Golgi_1; Golgi_2; Golgi_3; Golgi_4; Golgi_5]

Load this data into your program. In each of these experiments, intact cells have been loaded with pH fluorescent dyes that localize to specific organelles in the cell. The pH of the organelle can then be measured by recording the light emitted from the cell. At time zero, the cell was washed with a drug, bafilomycin, that inhibits the proton pump so that the organelles can no longer maintain their acidity, and they begin to alkalinize. This can be seen in the data sets.

5. For each experimental curve, begin with the initial pH near the pH of the first data point. Fit the model to the data using the curve fit procedure. Allow the program to adjust the proton permeability and the donnan particle concentration, B. Record the best-fit proton permeability. Remember that the proton pumps have been “turned off” experimentally. This means that N_pump=0 in your model. Repeat this for all the data sets and compute the mean and standard deviation for each organelle. Do these experiments show a noticeable difference in the bilayer leakiness between the Golgi and secretory granule?

NOTE: Remember to use the correct parameters from Table 1 when analyzing different organelles.

References

Grabe, M. and G. Oster (2001). Regulation of organelle acidity. J. Gen. Physiol.(In Press).

Rybak S, Lanni F, Murphy R. 1997. Theoretical Considerations on the Role of Membrane Potential in the Regulation of Endosomal pH. Biophys. J. 73(August 1997):674-687.

Tables

Parameter	Golgi	Secretory Granule	Endosome (MVB)
Surface Area [cm^2]	5.14×10^{-6}	1.26×10^{-9}	1.36×10^{-8}
Volume [L]	2.6×10^{-14}	4.2×10^{-18}	1.5×10^{-16}
Potassium permeability [cm/s]	1×10^{-5}	1×10^{-5}	1×10^{-5}
Buffering capacity [M/pH]	0.026	0.02	0.04

Table 1. Typical values for Golgi, secretory granules, and endosomes.

Parameter	Value
Cytoplasmic pH	7.4
Cytoplasmic potassium [M]	0.140
Membrane capacitance [kF/cm^2]	1×10^{-9}
Faraday's Constant [moles/Coulomb]	96,480
Avegadro's Number [molecules/mole]	6.02×10^{23}
F/(RT) [mV^{-1}]	$(25.69)^{-1}$

Table 2. Constants and typical variables.