The steady state kinetics of ATP synthesis and hydrolysis catalyzed by the chloroplast dicyclohexylcarbodiimide-sensitive ATPase reconstituted into phospholipid vesicles were studied as a function of the pH gradient. Bacteriorhodopsin also was incorporated into the vesicles so that a constant pH gradient could be maintained by continuous illumination of the liposomes. The dependence of the initial rates of ATP synthesis and hydrolysis on substrate concentrations is consistent with Michaelis-Menten kinetics, with enzyme, ADP, and P$_i$ forming a ternary complex. The Michaelis constants for both synthesis and hydrolysis are essentially independent of the pH gradient, while the maximum velocities depend strongly on it. The equilibrium constant for hydrolysis was calculated from the steady state kinetic parameters, and the dependence of the equilibrium constant on the pH gradient indicates that 3 protons are transported per ATP synthesized or hydrolyzed. The dependence of the steady state kinetic parameters on the pH gradient can be described by a mechanism in which the binding of substrates occurs before the transport of protons and the transport of the 3 protons is sequential rather than concerted.

The demonstration of reversible proton translocating activity of purified ATPases reconstituted into phospholipid vesicles has provided strong support for the chemiosmotic hypothesis (1,2). Although little doubt remains about the ability of a chemiosmotic gradient to drive ATP synthesis in these systems, considerable controversy exists with regard to the mechanism of this process (3). Much of the experimental work has been done on chloroplast or mitochondrial systems. To avoid some of the complexities introduced by these heterogeneous systems, we have studied the catalytic properties of the purified dicyclohexylcarbodiimide-sensitive ATPase from chloroplasts reconstituted into phospholipid vesicles. A constant transmembrane proton gradient was achieved by also incorporating bacteriorhodopsin into these vesicles; upon illumination the vesicles pumpe protons into the vesicles and creates a pH gradient.

The steady state kinetic parameters for both ATP hydrolysis and synthesis were determined as a function of the pH gradient, $\Delta$H. The equilibrium constant for ATP hydrolysis was calculated from the kinetic parameters by use of the Haldane relationship. The dependence of the equilibrium constant on $\Delta$H can be used to determine the stoichiometry of protons transported per ATP hydrolyzed or synthesized, while the dependence of the kinetic parameters on $\Delta$H can be interpreted in terms of a specific mechanism.

**EXPERIMENTAL PROCEDURES**

Octylglycoside (octyl-$\beta$-D-glucopyranoside) was purchased from Calbiochem. Hexokinase, ATP, ADP, 8-anilino-1-naphthalene sulfonate, valinomycin, and 9-aminoacridine were obtained from Sigma and chlorophenol red was from Fisher Scientific Co. The [y-$^3$P]ATP was purchased from New England Nuclear. The ATP was further purified by loading it on a 5-cm Bio-Rad AG 1- X4 column, washing with water, and washing with 10 mM HCl, 50 mM NaCl to remove AMP and ADP. The ATP was eluted with 20 mM HCl, 500 mM NaCl, neutralized with 1 M Tris base and precipitated with 2 M barium acetate. The precipitate was solubilized by exchanging the barium for lithium using a slurry of Bio-Rad AG50W-X8 Li$^+$ resin. Tl$^{3+}$[P]P phosphate was obtained from ICN and was heated at 50-100 °C in 2 n HCl for 1 to 2 h to ensure hydrolysis of any pyrophosphate. The asolectin (96% purified soybean phospholipid) was from Associated Concentrates Inc. All other reagents were of high grade commercial quality. Solutions were made with deionized distilled water.

Cell suspensions of Halobacterium halobium S-8 were a gift from Prof. Russell MacDonald. Bacteriorhodopsin was purified from these suspensions using a sucrose step gradient (4). The concentration of bacteriorhodopsin was determined by the absorbance at 560 nm assuming an extinction coefficient of 54,600 $M^{-1}cm^{-1}$ and a molecular weight of 26,000. The enzyme was prepared from market spinach by a modification (5) of the procedure of Pick and Racker (6). Concentrations of enzyme were determined by a modified Lowry procedure (7). The preparation of enzyme is approximately 27% pure (5), and this factor was used in calculating specific activities.

Vesicles were made by sonicating asolectin (40 mg/ml) to clarity in 0.15 M KCl, 50 mM Na-Tricine pH 8.0, and 1 mM EDTTA. Bacteriorhodopsin (0.5 or 1.0 mg/ml) was reconstituted into the vesicles (20 mg of asolectin/ml) by incubation on ice for 10 min in the presence of 1.25% octylglucoside. To remove the octylglucoside the vesicles were diluted 1:25 with buffer and centrifuged for 30 min at 40,000 rpm in a Beckman 60 Ti rotor. The pellet was taken up in buffer to give an asolectin concentration of ~40 mg/ml. The enzyme (0.5 or 1.0 mg/ml) was reconstituted into these vesicles by the freeze-thaw technique (8). ATP hydrolysis and synthesis rates were determined using standard radiochemical assays measuring the amount of organic $^{32}$P (synthesis) or $[^{33}]$P (hydrolysis) (9). Valinomycin (final concentration 1 $\mu$m) was added to the assay mixture to eliminate a membrane potential. All assays were carried out in buffer with 5 mM MgCl$_2$ in excess of the magnesium bound by the substrate. The binding constants for Mg$^{2+}$ to ATP, ADP, and P$_i$ were assumed to be 2.0 × 10$^3$, 2.2 × 10$^5$, and 3.2 × 10$^4$ M$^{-1}$, respectively, in calculating the free Mg$^{2+}$ concentration (10). The reconstituted vesicles were diluted 1:10 in the assay. For the synthesis assays, 10 units of hexokinase and 5 mM glucose were also present. The extent of reaction was determined at 0, 5, 10, and 15 min in kinetic experiments, and the slope of the linear plot of $^{[33]}$P$^{(3)}$, (hydrolysis) or $[^{32}]$P$^{(4)}$, (synthesis) $versus$ time was taken as the initial velocity. The variation of the maximum velocities with $\Delta$H was measured on a single reconstitution within the same day. For determination of the Michaelis constants, the abbreviation used is: Tricine, N-tris(hydroxymethyl)methylglycine.

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results on different days or preparations were normalized to give the same maximum velocity. All experiments were done at room temperature which was 22 ± 2°C.

In the kinetic experiments, samples were illuminated with a GE ELH 300-watt lamp and a 3-inch focal length lens. This light was filtered with Corning CS 3-69 and 1-75 filters and a 1-inch pathlength of saturated aqueous copper sulfate to limit the spectral region of illumination to 520 to 725 nm. Light intensities were varied using Ditrice neutral density filters. The pH gradient across the vesicles was monitored by the quenching of the fluorescence of 9-aminoacridine which was at a concentration of 5.0 μM in the assay (11). The fluorescence was detected with a Ditrice 480 nm narrow bandpass filter and an EMI 9635QB photomultiplier. The fluorescence was excited at 385 nm with a Hanovia 200-watt Xenon arc lamp and a 0.25-m Bausch and Lomb monochromator.

The fraction of volume in the vesicles was determined by forming the vesicles in the presence of 1 mM chlorphenol red. The external chlorphenol red was removed by passing the vesicles through a Sephadex G-50 column (1.6 cm inside diameter × 5 cm). The fraction of volume in the vesicles is the ratio of the absorbance at 590 nm of the final vesicle solution to that of the original vesicle solution with appropriate correction for the volume change occurring on passage through Sephadex. The absorbances were corrected for light scattering contributions by measuring the spectra of vesicles without dye at the same concentration as the vesicles with dye. As a further check, the internal contents of the vesicles (after passage through Sephadex) were exchanged with the external volume by repeated freeze-thaws, and the vesicles were removed by passage through a Millipore filter. The absorbance of this solution was within 5% of the absorbance of the solution with vesicles prior to the freeze-thaw procedure.

RESULTS

The reconstituted enzyme-phospholipid vesicles are extremely leaky to protons. This is evidenced by the lack of formation of a proton gradient upon ATP hydrolysis and by the inability of the vesicles to maintain a pH gradient after a pH jump. Although this has the disadvantage of providing low levels of ATP synthesis, it has the major advantage of providing a pH gradient that is determined by the bacteriorhodopsin pumping rate and the passive leak rate rather than by the proton-translocating activity of the enzyme. Thus, ΔpH is constant and independent of substrate concentrations. The absence of a membrane potential was ascertained by measurements of the fluorescence of 8-anilino-1-naphthalene sulfonic acid during the assay (12).

The pH gradient was calculated from the relationship (13)

\[ \Delta pH = \log(Q/(1-Q)V) \]  

where Q is the fraction of fluorescence quenched upon forming a pH gradient and V is the ratio of the internal vesicular volume to the total volume of the solution. Although the general validity of this equation has been questioned (14), it can be considered to be a good empirical estimate of ΔpH in liposomes (13). From internal volume determinations on several preparations, V was found to be 0.0117 (± 0.0006). (The internal volume is approximately 3 μl/mg of phospholipid.)

The steady state kinetics at constant ΔpH are considered first. The hydrolysis reaction follows simple Michaelis-Menten kinetics at all values of ΔpH studied. The kinetic parameters were determined from a least squares analysis of plots of (ATP)/υhyd versus (ATP) where υhyd is the steady state initial velocity. Such plots are shown in Fig. 1 for ΔpH = 0 and 2. The common intercept of the two lines on the abscissa indicates the Michaelis constant for ATP, KATP, is independent of ΔpH.

The rate law found for the steady state velocity of the synthesis reaction, υsyn is

\[ υ_{syn} = \frac{V_{max}}{1 + K_{ATP}/(ATP) + K_{ADP}(ADP) + K_{Pi}(Pi)} \]

where \( V_{syn} \) is the maximum velocity for synthesis and the \( K_i \) are Michaelis constants. Plots of (ADP)/υsyn versus (ADP) at constant Pi concentrations are linear; some typical data are shown in Fig. 2. The data were fit to equation 2 by a nonlinear least squares analysis. The Michaelis constants obtained are summarized in Table I, along with the Michaelis constants for ATP. The uncertainty in these parameters is about ±20%.

The simplest mechanism for ATP hydrolysis and synthesis at constant ΔpH consistent with the steady state data is

\[ ATP + E \rightleftharpoons E-ATP \]
\[ \rightleftharpoons E-ADP+P \]
\[ \rightarrow E-ADP+Pi \]
\[ \rightarrow E-P+Pi \]
\[ \rightarrow E-P+ADP \]

\[ \rightarrow E-P+Pi \]

The data cannot distinguish between a mechanism in which P and ADP equilibrate rapidly with the enzyme prior or subsequent to the rate-determining step and one in which ADP and P combine with the enzyme and are released in a defined sequential order. The addition of P and ADP is not completely random in any event since \( K_{AP} > K_{P} \) is not.

The maximum velocities were determined as a function of ΔpH by measuring the initial velocity for hydrolysis at an ATP concentration of 3.0 mM and that for synthesis at ADP and Pi concentrations of 30 μM and 10 mM, respectively. The
Kinetics of ATP Synthesis and Hydrolysis

The internal hydrogen ion concentration to the hydrogen concentration outside the phospholipid vesicles, \((H^+)/[H_+]^1\). Open symbols (left ordinate) are hydrolysis and closed symbols (right ordinate) are synthesis. The reconstituted protein concentration in the assay is 0.05 mg/ml of enzyme, 0.20 mg/ml of bacteriorhodopsin (C); 0.10 mg/ml of enzyme, 0.20 mg/ml of bacteriorhodopsin (C); and 0.05 mg/ml of enzyme, 0.10 mg/ml of bacteriorhodopsin (Δ). The lines are the best fits to equations 6 and 7 with the parameters given in the text. The experimental conditions are given in the legend to Fig. 1.

Fig. 4 (right). Plot of the logarithm of the equilibrium constant, \(K_{\text{app}}\), for the hydrolysis of ATP versus \(\Delta pH\). The reconstituted protein concentration in the assay is 0.05 mg/ml of enzyme, 0.20 mg/ml of bacteriorhodopsin (C); 0.10 mg/ml of enzyme, 0.20 mg/ml of bacteriorhodopsin (C); and 0.05 mg/ml of enzyme, 0.10 mg/ml of bacteriorhodopsin (Δ). The experimental conditions are given in the legend to Fig. 1.

### TABLE I

| Michaelis constants for ATP synthesis and hydrolysis at different values of \(\Delta pH\) |
|------------------|------------------|
| \(\Delta pH\)    | \(K_{\text{ATP}}\) (mM) | \(K_{\text{ATP}}\) (mM) | \(K_{\text{E}}\) (mM) | \(K_{\text{DOP}}\) (10^9 M\(^{-2}\)) |
| 0                | 0.40             | 1.5                | 0.11               | 1.35             |
| 1.92             |                  | 2.4                | 0.15               | 0.96             |
| 2.00             |                  |                    |                    |                  |

maximum velocities, which were only a few per cent more than the initial velocities, were calculated by use of the known substrate Michaelis constants. The results obtained are shown in Fig. 3. The uncoupled ATPase activity has been subtracted from the measured maximum velocity. It was determined by extrapolating the measured velocities to infinite \(\Delta pH\). The dependence of the maximum velocities on \(\Delta pH\) was identical when the enzyme concentration in the reconstitution procedure was 0.5 and 1 mg/ml. However, the uncoupled activity was about 40% of the dark ATPase activity in the former case and 75% in the latter case. Obviously reconstitution is much more efficient at the lower enzyme concentration.

The overall reaction for ATP hydrolysis and proton pumping at constant outside pH can be written as

\[
\text{ATP} + nH^+ \rightarrow ADP + P_i + nH_2O
\]  

where \(o\) and \(i\) designate the proton concentrations outside and inside the vesicles. The equilibrium constant for this reaction, \(K_{eq}\), can be expressed in terms of the steady state kinetic constants as

\[
K_{eq} = \frac{(ADP)(P_i)[(H_2O)(H_2O)]}{(ATP)[(H_2O)]^{n}}
\]

The apparent equilibrium constants calculated from the kinetic parameters, \(K_{\text{app}}\), are shown as a function of \(\Delta pH\) in Fig. 4. In calculating the equilibrium constants, the average values of \(K_{\text{ADP-F}}\) and \(K_{\text{ATP}}\) were used since they do not appear to depend significantly on \(\Delta pH\). A least squares analysis of the data in Fig. 4 gives a value for \(n\) of 3.0 and a value for the equilibrium constant of 7 at \(\Delta pH = 0\). These values do not depend strongly on the value chosen for the uncoupled ATPase activity. If 10 specific activity units are subtracted from the values of \(V_{\text{hyd}}\) shown in Fig. 4, \(n = 3.4\) and \(K_{eq} = 16\) when \(\Delta pH = 0\). If 40 units are added to the values of \(V_{\text{hyd}}, n = 2.6\) and \(K_{eq} \approx 2\) when \(\Delta pH = 0\).

### DISCUSSION

The mechanism proposed for ATP hydrolysis and synthesis at constant \(\Delta pH\) (Equation 3) is the simplest consistent with the data. No attempt has been made to differentiate between mechanisms involving sequential addition of ADP and P\(_i\) and the rapid equilibration of ADP and P\(_i\) with the enzyme. While this could be readily done by the use of nucleotide inhibitors for a simple reaction, the allosteric effects of nucleotides is likely to yield ambiguous results in this case. Recent experiments with thylakoids suggest an ordered mechanism with ADP binding first (15), but this conclusion is based on a complex mechanism. The Michaelis constant for ADP, 2 μM, is similar to the dissociation constant obtained from equilibrium binding measurements (5) but is quite different from that reported for thylakoids (85 μM, Ref. 15). The reason for this discrepancy is not known. However, the fact that dithiothreitol activation of the enzyme occurs during preparation may be significant. An alternating site mechanism has been proposed for ATP-synthesizing enzymes (3), and many variations of such a mechanism also can give the rate laws observed in this study. However, the Haldane relationship remains the same in these cases so that the calculation of the equilibrium constant from the kinetic parameters is independent of the detailed mechanism. A major concern in using a
reconstituted system is that the specific activity for synthesis is less than 1% of that estimated for chloroplasts (16). Part of this difference probably is due to the larger pH gradient obtained with chloroplasts, but further improvements in the enzyme purification and reconstitution are needed.

The dependence of the equilibrium constant on ΔpH indicates 3 protons are pumped per ATP synthesized or hydrolyzed. Previous determinations of this stoichiometry have been done primarily on mitochondria and chloroplast thylakoid preparations (17). The method used in this work, namely measurements of initial steady state rates for both hydrolysis and synthesis, has not been utilized previously. Results on mitochondrial and bacterial chromatophores have been variable, but a stoichiometry of three often has been reported with chloroplasts (17, 18). The most serious error in the determination of the proton-pumping stoichiometry in this work is in measuring ΔpH. In fact, we require only that the relative values of ΔpH be known to obtain the correct stoichiometry. The fact that the value of 4 when ΔpH = 0 is about 10 m rather than the known value of 5 × 10 m under similar conditions (pH 8.0, 5 mM Mg2+, 0.2 m ionic strength) (19) suggests that the effective value of ΔpH is considerably larger than calculated. A possible source of error in determining ΔpH is measurement of the internal volume of active vesicles. The method we have used determines the total internal volume, which clearly overestimates the value of V which should be used in Equation 2. The result is that ΔpH is probably underestimated by a constant increment. Also from a simple statistical argument given under “Appendix,” ΔpH is underestimated if a population of large vesicles with large pH gradients is present. While vesicle heterogeneity and statistical anomalies in the distribution of enzyme and bacteriorhodopsin are potential problems, the same dependence of the kinetic parameters on ΔpH is observed at two different concentrations of enzyme and bacteriorhodopsin. This suggests these problems are not causing anomalous results, except perhaps for a systematic error in ΔpH.

While the Haldane relationship does not depend on mechanistic details, nucleotide regulation could cause an error in the calculated values of the apparent equilibrium constant. For example, if ADP and/or F1 convert the enzyme to a more active form and ATP to a less active form, then steady state parameters of essentially different enzymes would be determined in the synthesis and hydrolysis experiments. Efforts to find such regulation have been unsuccessful thus far, but this possibility merits further consideration. This mechanism makes good sense physiologically: the enzyme is most efficient when the ATPase activity is suppressed by the pH gradient and ATP is synthesized; when high concentrations of ATP are present and the pH gradient is absent, the enzyme would be inefficient, thus conserving ATP.

A number of mechanisms have been examined to explain the dependence of the steady state kinetic parameters on ΔpH. The most significant finding is that the Michaelis constants on ΔpH has been observed. This result has two important mechanistic implications: 1) proton pumping must occur after the binding of substrates; and 2) a concerted transport of 3 protons is not possible. We have been unable to find any mechanisms without either of the above features that have the Michaelis constants independent of ΔpH and the maximum velocities strongly dependent on ΔpH.

A mechanism consistent with the data is presented in Fig. 5. In this mechanism, two discrete proton transfer events occur when ATP or ADP and P, are bound to the enzyme; 2 protons are transferred in the first event and one in the second. A mechanism in which three discrete proton-pumping steps occurs also is possible but has more adjustable parameters. For the sake of simplicity, the equilibration of ADP and P, with the enzyme is assumed to be rapid relative to hydrolysis/synthesis and proton pumping, but this is not a necessary requirement. The step at which hydrolysis/synthesis occurs is not specified since this cannot be assessed from the data. If 4 > H+, the dependence of the steady state kinetic parameters on H+ is given by the following equations:

\[
V_{\text{syn}} = \frac{C_0}{1 + C_1 (H^+)/ (H^+)^2} \\
K_{\text{ADP}} = K_0 \\
K_{\text{ADP}} = K_0 \\
K_{\text{ADP}} = C_1 + C_3 (H^+)^2 \\
K_{\text{ADP}} = C_1 + C_3 (H^+)^2
\]

The C0, which are functions of the rate and equilibrium constants and of (H+), are defined under “Appendix” where the exact kinetic equations for the mechanism are given. The dependence of V1 and V2 on ΔpH were fit to Equations 6 and 7 by a nonlinear least squares analysis. The lines in Fig. 3 represent the best fits which were obtained with C0 = 139 activity units, C1 = 6.0 × 10−3, C2 = 1.2 × 10−4 activity units and C3 = 7.9 × 10−6. As observed, KADP, KADP, and KADP are independent of H+. In order for KTP to be independent of H+, as observed, C1 and C2 must be equal. The data-fitting procedure shows C1 = C2; an analysis of the equations under “Appendix” shows that C1 = C2 if k2 > k3 (H+) and k2 < k3. If C1 is set equal to C2 in the data analysis, the dependence of the maximum velocities on ΔpH is fit approximately as well as in the original fitting procedure. Thus the experimental data are consistent with the mechanism in Fig. 5.

The mechanism in Fig. 5 cannot be taken literally but is sufficient to explain the data. In summary, the results appear

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3 Recent results indicate that because of the dependence of the observed fluorescence quenching on the 9-aminoacridine and vesicle concentrations, ΔpH has been underestimated by about 1 pH unit in this work. This would result in K0m = 10 m at ΔpH = 0.

---

Fig. 5. Proposed mechanism for proton pumping and ATP synthesis and hydrolysis in reconstituted chloroplast coupling factor. The upper case Ks are equilibrium constants and the lower case ks are rate constants. The Xs are reaction intermediates.
to require that proton pumping occurs after the binding of substrates and that the 3 protons are not pumped in a single step. In the specific mechanism presented, the pumping of 2 protons from the outside to the inside occurs after the binding of ATP; the resulting intermediate then pumps an additional proton. The hydrolysis or synthesis of ATP could occur concurrently with a pH-independent Michaelis constant for ATP, C in equations 6 and 7. This requires that the average pK of the groups transporting the proton on the outside releasing a proton must be less than 6 ($k_2 \gg k_3 (H_+)^-)$).

The finding of tight proton binding on the ionizable group is expected. These pK values are derived from a quantitative fitting of the pH dependence of the kinetic parameters to this particular mechanism; therefore, they should be regarded as qualitative assessments of the pK values. This mechanism is valid even if a systematic error in ΔpH exists; this can be accommodated by a simple scaling of the kinetic parameters.

Postulation of a molecular mechanism for proton pumping in term of molecular structure falls into the realm of speculation. Nevertheless, the simplest type of mechanism to envisage is one in which conformational changes triggered by substrate binding and/or synthesis and hydrolysis alter the environment of ionizable groups as to change the associated pK values from $>8$ to $<6$ as the ionizable groups are exposed to the outside and inside, respectively. Such a mechanism is pictured schematically in Fig. 6 for the pumping of a single proton. In this mechanism, the binding of ATP triggers a conformational change which exposes the ionizable group to the inside previously exposed to the outside, the pK is then lowered, a proton is lost to the inside, the ionizable group is protonated, and ADP and P$_i$ are dissociated. The hydrolysis step is intentionally not specified. The changes in pK can be attributed to changes in the environment of the ionizable group. This mechanism is reversible, with proton pumping then occurring from the inside to the outside. Extension of such a mechanism to more than a single proton is obvious.

The work represents a first attempt to establish a molecular mechanism for the coordination of proton pumping and ATP hydrolysis and synthesis. We are extending this approach to consider variations in the external pH, the metal ion concentration, and the membrane potential and to improve the measurement of the effective ΔpH.

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APPENDIX

Dye Quenching from a Heterogeneous Population of Vesicles

Assume a population of vesicles with subpopulations (designated with a subscript $k$) which have discrete distributions with respect to the internal volume fraction, $V_k$, and internal hydrogen ion concentration, $(H_+)_k$. Following Schindler (11), the relationship between hydronium ion concentration, internal volume fraction, and fluorescence, $F_k$, for a subpopulation is:

$$\frac{(H_+)_k}{(H_+)} = \frac{F_k}{F_k F_0 - F_k}$$

where $F_k$ is the fluorescence with no pH gradient and should be the same for all populations. The total observed fluorescence is:

$$F = \sum_k f_k F_k$$

where $f_k$ is the fraction of the total population that is in the $k$th subpopulation. The experimentally observed internal hydrogen ion concentration, $(H_+)_obs$, will then be:

$$\frac{(H_+)_obs}{(H_+)} = \frac{1}{\sum_k f_k (H_+)_k} \frac{1}{F_0 - F} = \frac{1}{\sum_k f_k (H_+)_k} \frac{1}{F_0 - F}$$

The $(H_+)_k$ term in the denominator of the summations will have the effect of making $(H_+)_obs$ smaller than the average internal hydrogen ion concentration. For instance, consider a population where one-tenth of the vesicles have $V = 10^{-3}$ and $(H_+)_k = 10^{-5}$ m and nine-tenths of the vesicles have $V = 9 \times 10^{-3}$ and $(H_+)_k = 10^{-6}$ m for $(H_+)_obs = 1.9 \times 10^{-6}$ m.

Kinetic Mechanism

The steady state kinetic parameters derived from the mechanism in Fig. 5 are:

$$V_{cat} = \frac{k_1 k_2 k_3 (H_+)^3}{(H_+)^2 [k_2 k_3 (H_+)^2 + k_3 k_0 (H_+)^3] + (H_+)^3 [k_3 k_2 + k_1 k_0 (H_+)^3]}$$

$$K_{ATP} = \frac{k_5 (H_+)^2}{k_1 (H_+)^2 [k_5 k_0 (H_+)^2 + k_3 k_0 - k_1 k_0 (H_+)^3] + k_4 (H_+)^3 [k_3 k_2 + k_1 k_0 (H_+)^3]}$$

$$V_{syn} = \frac{k_6 k_7 k_8 (H_+)^3}{(K_0 k_7 (H_+)^2 + k_8 k_0 - k_1 k_0 (H_+)^3) + k_9 (H_+)^3 [k_3 k_2 + k_1 k_0 (H_+)^3]}$$

$$K_{ADP,F} = \frac{k_1 k_9 (H_+)^3}{k_2 k_0 (H_+)^2 + k_3 k_0 - k_1 k_0 (H_+)^3 + k_4 k_0 - k_1 k_0 (H_+)^3}$$

$K_0 k_7 (H_+)^2 + k_8 k_0 - k_1 k_0 (H_+)^3 + k_9 (H_+)^3 [k_3 k_2 + k_1 k_0 (H_+)^3]$
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\[ K_{\text{ADP}} = \frac{K_{\text{ADP}}}{K_7} \]

\[ K_P = \frac{K_{\text{ADP}}}{K_8} \]

If \( K_5 \gg (H_\text{+]}) \), the steady state parameters are given by Equations 6 to 11 with

\[ C_0 = \frac{k_3 k_4 k_5 (H_\text{+]})^2}{k_3 k_4 + k_{-2} k_4 + k_2 k_3 (H_\text{+]}) + k_3 k_4 (H_\text{+]})^2} \]

\[ C_1 = \frac{[k_2 k_3 + k_2 k_4 (H_\text{+]})^2]}{[k_4 + k_{-2} k_4 + k_3 k_4 (H_\text{+]}) + k_3 k_4 (H_\text{+]})^2]} \]

\[ C_2 = \frac{k_{-1} k_{-2} k_{-3} k_{-4} (H_\text{+]})^2}{k_{-1} k_{-2} k_{-3} k_{-4} (H_\text{+]})^2 + k_{-5} k_3 + k_{-1} k_{-2}} \]

\[ C_3 = k_4 C_2 / k_{-4} (H_\text{+]}) \]

\[ K_{\text{ADP},P} = K_6 K_7 \]

\[ K_{\text{ATP}} = C_1 \left[ \frac{1 + C_2 [(H_\text{+]})/(H_\text{+[+]})^2]}{1 + C_2 [(H_\text{+]})/(H_\text{+[+]})^2]} \right] \]

\[ C_4 = \frac{k_4 k_5 k_6 (H_\text{+[+]})^2 + k_{-4} k_4 + k_{-2} k_4}{k_4 k_5 k_6 (H_\text{+[+]})^2} \]

\[ K_{\text{ADP}} = K_8 \]

\[ K_P = K_6 K_7 / K_8 \]

Note that \( C_1 = C_2 \) if \( k_{-2} \gg k_2 (H_\text{+[+]})^2 \) and \( k_3 < k_{-1}, k_{-2}, k_4 \).

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