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ORIGINAL PAPER

Is the Ca^{2+}-ATPase from sarcoplasmic reticulum also a heat pump?

Signe Kjelstrup · Leopoldo de Meis · Dick Bedeaux · Jean-Marc Simon

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Abstract We calculate, using the first law of thermodynamics, the membrane heat fluxes during active transport of Ca^{2+} in the Ca^{2+}-ATPase in leaky and intact vesicles, during ATP hydrolysis or synthesis conditions. The results show that the vesicle interior may cool down during hydrolysis and Ca^{2+}-uptake, and heat up during ATP synthesis and Ca^{2+}-efflux. The heat flux varies with the SERCA isoform. Electroneutral processes and rapid equilibration of water were assumed. The results are consistent with the second law of thermodynamics for the overall processes. The expression for the heat flux and experimental data, show that important contributions come from the enthalpy of hydrolysis for the medium in question, and from proton transport between the vesicle interior and exterior. The analysis give quantitative support to earlier proposals that certain, but not all, Ca^{2+}-ATPases, not only act as Ca^{2+}-pumps, but also as heat pumps. It can thus help explain why SERCA 1 type enzymes dominate in tissues where thermal regulation is important, while SERCA 2 type enzymes, with their lower activity and better ability to use the energy from the reaction to pump ions, dominate in tissues where this is not an issue.

Keywords Ca^{2+}-ATPase · Active transport · Heat transport · Ion pump

Signe Kjelstrup and Dick Bedeaux on leave from Department of Chemistry, Norwegian University of Science and Technology, NO-7491, Trondheim, Norway. Jean-Marc Simon on leave from Institut Carnot de Bourgogne, UMR 5209 CNRS-Université de Bourgogne, F-21078, Dijon Cedex, France.

S. Kjelstrup (✉) · D. Bedeaux · J.-M. Simon
Centre of Advanced Study, Norwegian Academy of Science and Letters, Drammensveien 78, 0271 Oslo, Norway
e-mail: signe.kjelstrup@chem.ntnu.no

L. de Meis
Instituto de Bioquímica Medica, Prédio CCS, Universidade Federal do Rio de Janeiro, Cidade Universitária, RJ 2194-590, Brazil

Introduction

The Ca^{2+}-ATPase from sarcoplasmic or endoplasmic reticulum (SERCA) can transport Ca^{2+}-ions against their concentration gradient. Energy is then supplied by the spontaneous reaction of adenosine triphosphate (ATP) into adenosine diphosphate (ADP) and inorganic phosphate (Pi). This is called active ion transport. SERCA isoforms that can be obtained from various tissues, vary in their ability to transport calcium and produce heat. This has been found by studying the performance of the various isoforms embedded in SERCA vesicles (de Meis 2002, 2003; Arruda et al. 2003).

The laws of thermodynamic are central for a description of all systems where energy conversion take place, thus also of biological systems, see, e.g., Tinoco (1995). The laws are needed to examine the conversion of energy into heat and work. The second law can be used to determine the spontaneous direction of processes, their rates and the energy dissipation (Caplan and Essig 1983; Kjelstrup et al. 2005), but a first law analysis is first needed to account for energy conservation.

The aim of this work is to analyze by means of the first law of thermodynamics experimental data on heat production (de Meis 2002, 2003; Arruda et al. 2003) from the operation of the Ca^{2+}-ATPase in vesicles from sarcoplasmic or endoplasmic reticulum. The results shall in the next round be used to give a basis for a detailed second law analysis.

The experiments referred to were done under two stationary state conditions, i.e., initial state experiments, and states at zero net uptake of Ca^{2+}. All changes per unit of time are constant in these states. The change in internal energy per unit of time of a system, dU/dt, is then the sum of the heat supplied to the system from the surroundings plus the work performed on the system per unit of time. When the pressure is constant this can also be written as

$$\frac{dH}{dt} = \frac{d}{dt}(U + pV) = \frac{dq}{dt} \quad (1)$$

where dH/dt is the rate of enthalpy change in the system. Some volume work or elastic deformation work is probably done during the enzyme cycle as suggested by structural findings (Olesen et al. 2007). This is included in the pV-term. Electric work shall be neglected, see below, Eq. (4).

The heat-producing enzymes are embedded in SERCA vesicles. A suspension of vesicles is added to a solution of known composition to start the experiment where dq/dt is measured. The suspension, but not necessarily the vesicle interior, is thermostated by the isothermal calorimeter that is used in the measurement.

The article is organised as follows: We describe first briefly the overall processes associated with active transport. The total system is divided into three subsystems, and we describe all of them separately by the first law. As subsystems, we consider the solution in which the vesicles are suspended, the vesicle membrane, and the vesicle interior, see Fig. 1. We shall find that the molecular pump is not only an ion pump, but, given that certain premises hold, also a heat pump. The heat pump capacity shall be calculated from published experimental data.

ATP conversion and Ca^{2+} transport

The hydrolysis reaction of ATP takes place in a solution which is buffered at a particular pH and a particular Mg^{2+}-concentration (pMg). The reaction is, according to convention (Alberty 2003), written without charges and cations bound:

$$ATP + H_2O \rightleftharpoons ADP + Pi \quad (2)$$

The enthalpy of the reaction is denoted by

$$\Delta H'_{hydr} = H_{ADP} + H_{Pi} - H_{ATP} - H_w \quad (3)$$

where H_i are partial molar enthalpies for the components i = ATP, ADP, Pi and water, at the pH, pMg, temperature, ionic strength, and buffer in use. When the species are in their standard state, the reaction enthalpy at 313.15 K, ionic strength 0.25 M, pH = 7.00 and pMg = 2 is −25.7 kJ/mol (Alberty 2003). A good estimate for $\Delta H'_{hydr}$ in the present situation, where pH and pMg have these values, is thus −26 kJ/mol. The proton production by Eq. 2 leads to buffer protonation, and this adds to the sensible heat change by the reaction, see Alberty (2003), de Meis et al. (2005) and Eq. (13) below.

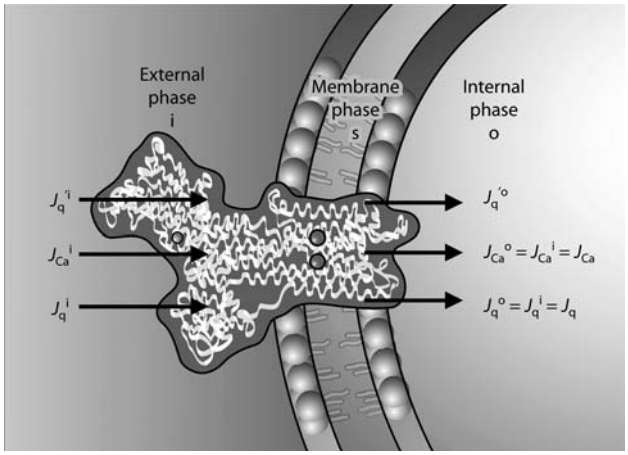

Fig. 1 Schematic illustration of experimental system inside the calorimeter. The external phase i, with the suspension of vesicles, reactants (ATP, water and Ca^{2+}) and products (ADP, Pi), the vesicle membrane phase (s) with the Ca^{2+}-ATPase and the vesicle interior phase o. Positive flux direction is defined from left to right. Conditions for mass and energy conservation at stationary state are given in the bottom right corner

The rate of formation of Pi defines the reaction rate, r. A typical unit for r is moles/mg min, where mg refers to the amount of Ca^{2+}-ATPase (catalyst). Isotope labeling is used to follow the breakdown or formation of ATP, so the experiment gives unidirectional fluxes. The net rate is the difference between the forward and reverse unidirectional rates; $r = r_{hydr} - r_{synth}$. Only the net rate, r, can be used in thermodynamic considerations.

The number of Ca^{2+} ions transported per turnover of ATP is characteristic for each SERCA Ca^{2+} -ATPase. There are two binding sites for Ca^{2+} on the Ca^{2+}-ATPase. A Ca^{2+}-ATPase which transports 2 Ca^{2+} per ATP turnover, is said to be coupled. In the coupled state of operation, one ATP leads to translocation of 2 Ca^{2+}. This value has only been found when the concentration of Ca^{2+} inside the vesicle is kept very low. When the Ca^{2+}-ATPase works in its uncoupled state, there is ATP hydrolysis, but no Ca^{2+}-uptake. The isoforms operate with a varying probability for Ca^{2+} to be transported. For instance, in vesicles from white skeletal muscle (SERCA 1), initial rates for ion transport and hydrolysis have a ratio between 0.3 and 0.5. The ion is less likely to be transported by ATP hydrolysis in SERCA 1 from white muscle, than in SERCA 2 from blood platelets and red muscle.

Obara et al. (2005) reasoned from structural considerations, that 2–3 H^+ are likely to move in the direction opposite to Ca^{2+}, possibly also with water(s) attached.

Hauser and Barth (2007) have identified four proton binding sites active in the translocation of protons. Karjalainen et al. (2007) proposed a proton path that is partly separate from the Ca^{2+}-path. Fibich et al. (2007) argued from electrostatic considerations that ion-binding sites are always occupied, and that two protons and one Ca^{2+}-ion replace each other. Net charge build-up is unlikely in the absence of red/ox reactions. We therefore propose that there is an exchange of ions, through one or more pathways, according to:

$$Ca_i^{2+} + 2H_o^+ \rightleftharpoons Ca_o^{2+} + 2H_i^+ \tag{4}$$

Reaction (4) means that we are in agreement with Eq. 1, no internal energy is used to do electric work. The enthalpy change of the exchange reaction is:

$$\Delta H_{Ca/2H} = H_{Ca}^o - H_{Ca}^i + 2H_H^i - 2H_H^o \tag{5}$$

The change in ion concentrations on the vesicle exterior (denoted i) or interior (denoted o), can also lead to reactions with buffers and chelators in the two solutions.

Similar to the situation for r, unidirectional rates are measured, following transport of ^{45}Ca (de Meis 2003). The net flux of Ca^{2+} into the vesicles is equal to: $J_{Ca} = J_{Ca}^{upt} - J_{Ca}^{eff}$where "upt" means uptake and "eff" means efflux (unit: moles/ mg min). The net flux is also proportional to the amount of catalyst.

There are water content and osmolarity changes by reaction (2), so we assume further that equilibrium can be re-established for water across the membrane:

$$H_2O(i) \rightleftharpoons H_2O(o) \tag{6}$$

Reaction (6) means that the change in osmolarity that follows from reaction (2) is accompanied by a flux of water from the vesicle interior to the exterior. Water transport might follow the proton transport in reaction (5) (Obara et al. 2005) or other routes. An active role for water at the catalytic site was already long ago suggested by de Meis and coworkers (1980, 1982).

The processes (2, 4 and 6) can be reversed. The hydrolysis of ATP can drive Ca^{2+}-uptake, but Ca^{2+}-efflux can also drive synthesis of ATP. The Ca^{2+} need not exit by the same path as they enter (de Meis 2001). In the case of so-called *uncoupled efflux*, Ca^{2+} is leaving the vesicle without making ATP.

The first law for ATP conversion and Ca^{2+} transport

The experimental system in question was divided into three subsystems plus surroundings, see Fig. 1. The calorimeter represents the surroundings. The solution in which the vesicles are suspended, are indicated by i in Fig. 1. The ensemble of membranes, that contains the Ca^{2+}-ATPases, is indicated by s. The vesicle interior is indicated by o. For all subsystems the first law is:

$$\frac{dH}{dt} = \frac{dq}{dt} = J_q^{\text{left}} - J_q^{\text{right}} \tag{7}$$

where J_q^{left}, J_q^{right} are total heat fluxes on the left hand side and right hand side of the subsystem, respectively. As positive direction we take the direction into the vesicles. The definition of the total heat flux for an open system is (Sandler 2006)

$$J_q = J_q' + \sum J_i H_i \tag{8}$$

The total heat flux J_q is the sum of the measurable heat flux, J_q', and the latent heats moving as enthalpies with the components. With the convention chosen for positive flux, the measurable heat change in the calorimeter is $-J_q^{\text{cal}}$. A typical unit is mJ/(mg Ca^{2+}ATPase) min. When a positive heat change is measured in the calorimeter, the processes in the system are exothermic, and $J_q < 0$. When a negative heat change is measured in the calorimeter, the processes are endothermic.

Subsystem: solution in which the vesicles are suspended

The first law for the solution in which the vesicles are suspended becomes, cf. Eq. (7):

$$\frac{dH^i}{dt} = J_q^{\text{cal}} - J_q^i \tag{9}$$

The heat flux from the calorimeter, J_q^{cal}, enters the solution by conduction through the walls, while the flux J_q^i is the total heat flux going into the membrane. At steady state, the composition changes in the solution are according to Eq. (2):

$$-\frac{dN_{\text{ATP}}}{dt} = \frac{dN_{\text{ADP}}}{dt} = \frac{dN_{\text{Pi}}}{dt} = r \tag{10}$$

where N_i is the amount of component i, in moles per mg of Ca^{2+}-ATPase. The reaction produces also an amount of protons, equal to $r\Delta_r N_H$ (Alberty 2003). The protons immediately reacts with the buffer in the solution.

The composition changes during hydrolysis define the following component fluxes in the membrane.

$$J_{\text{ATP}} = -J_{\text{ADP}} = -J_{\text{Pi}} = r \tag{11}$$

We assume that there is a flux of water related to removal of water by reaction (2):

$$J_w - \frac{dN_w}{dt} = 0 \tag{12}$$

The composition change in calcium ion in the external medium defines the fluxes of Ca^{2+} and H^+ according to reaction (4):

$$-\frac{\mathrm{d}N^i_{\mathrm{Ca}}}{\mathrm{d}t} = J_{\mathrm{Ca}} = -\frac{1}{2}J_H \tag{13}$$

Also the proton flux in the membrane, J_H, leads also to an immediate reaction with the buffer.

We can now examine the contributions to the enthalpy change $\mathrm{d}H^i/\mathrm{d}t$ in the i-phase. These follow from the composition changes, Eqs. (10, 12 and 13), plus the proton production by reaction 2, when the temperature is held constant. We obtain:

$$\begin{aligned}\frac{d\mathrm{H}^i}{\mathrm{d}t} &= H_{\mathrm{ADP}}\frac{\mathrm{d}N_{\mathrm{ADP}}}{\mathrm{d}t} + H_{\mathrm{Pi}}\frac{\mathrm{d}N_{\mathrm{Pi}}}{\mathrm{d}t} + H_{\mathrm{ATP}}\frac{\mathrm{d}N_{\mathrm{ATP}}}{\mathrm{d}t} \\ &\quad + H_w\left(\frac{\mathrm{d}N_{\mathrm{w}}}{\mathrm{d}t} - J_w\right) + H^i_{\mathrm{Ca}}\frac{\mathrm{d}N^{\mathrm{i}}_{\mathrm{Ca}}}{\mathrm{d}t} \\ &\quad + \left(\frac{\mathrm{d}N^i_{\mathrm{H}}}{\mathrm{d}t} + r\Delta_r N_H\right)\Delta H(\mathrm{buff}) \\ &= r\Delta H_{\mathrm{hydr}} - J_w H^i_w - J_{\mathrm{Ca}}(H^i_{\mathrm{Ca}} - 2H^i_H) \\ &\quad - (J_H + r\Delta_r N_H)\Delta H(\mathrm{buff})\end{aligned} \tag{14}$$

where $\Delta H(\mathrm{buff})$ is the enthalpy for buffer protonation, and the composition changes were replaced by the constant fluxes in the second line. The unprimed enthalpy of hydrolysis contains the primied quantity minus the term $r\Delta_r N_H H^i_H$.

On the other hand, we can introduce the definition (8) on the right hand side of Eq. (9). This gives:

$$\frac{\mathrm{d}H^i}{\mathrm{d}t} = J^{\mathrm{cal}}_q - J'^{,i}_q + r\Delta H_{\mathrm{hydr}} - J_w H^i_w - J^i_{\mathrm{Ca}}(H^i_{\mathrm{Ca}} - 2H^i_H) \tag{15}$$

Equations (14) and (15) describe the same enthalpy change, and we obtain:

$$J^{\mathrm{cal}}_q = J'^{,i}_q - (J_H + r\Delta_r N_H)\Delta H(\mathrm{buff}) \tag{16}$$

The measurable heat flux coming from the calorimeter, is the heat flux going into the membrane minus the heat change due to the shift in the buffer equilibrium. This relation gives an important first step for an interpretation of calorimetric experiments on the system. As we shall see below, the measurable heat flux into the membrane contain as a major term the enthalpy of hydrolysis.

Subsystem: membrane with Ca^{2+}-ATPase

Neither mass, nor energy or work accumulate in the membrane phase in the stationary state on the time scale of the experiment. Conservation of mass gives a constant flux of Ca^{2+} through the membrane, J_{Ca}, and of water, where $J_w = r$. Conservation of energy means that

$$J^i_q = J^o_q \tag{17}$$

The fluxes that describe mass and energy conservation are also given in the bottom right corner of Fig. 1. By using Eq. (17) with the definition (8), we obtain:

$$\begin{aligned}&J'^{,i}_q - r\Delta H_{\mathrm{hydr}} + J_{\mathrm{Ca}}(H^i_{\mathrm{Ca}} - 2H^i_H) + J_w H^i_w \\ &\quad = J'^{,o}_q + J_{\mathrm{Ca}}(H^o_{\mathrm{Ca}} - 2H^o_H) + J_w H^o_w\end{aligned} \tag{18}$$

or

$$J'^{,i}_q = J'^{,o}_q + r(\Delta H_{\mathrm{hydr}} + \Delta H_w) + J_{\mathrm{Ca}}\Delta H_{\mathrm{Ca/2H}} \tag{19}$$

where $\Delta H_w = H^i - H^o$ is the enthalpy difference of water between the vesicle exterior and interior. While the total energy flux through the membrane is constant, the measurable heat fluxes are not. Their difference is described by Eq. (19), as the enthalpy of hydrolysis of water transport and of ion exchange; in short by the enthalpy change of all processes (2, 4, and 6) that are associated with active ion transport.

Equation (19) gives a further interpretation of the calorimetric experiments

$$\begin{aligned}J^{\mathrm{cal}}_q &= J'^{,o}_q + r(\Delta H_{\mathrm{hydr}} + \Delta H_w) + J_{\mathrm{Ca}}\Delta H_{\mathrm{Ca/2H}} \\ &\quad - (J_H + r\Delta_r N_H)\Delta H(\mathrm{buff})\end{aligned} \tag{20}$$

The heat change measured in the calorimeter is, according to this equation, due to the hydrolysis of ATP, water equilibration, plus (if relevant) an exchange of Ca^{2+}-ions with protons and the accompanying shift in the buffer equilibrium. In addition, there is a heat flow into the vesicle interior that must be accounted for, see below.[1]

Subsystem: vesicle interior solution

The first law for the vesicle interior is according to Eq. (7)

$$\frac{\mathrm{d}H^o}{\mathrm{d}t} = J^o_q = J'^{,o}_q + J_{\mathrm{Ca}}(H^o_{\mathrm{Ca}} - 2H^o_H) + J_w H^o_w \tag{21}$$

The vesicle inside has no direct contact with the thermostat (the calorimeter) and might well have a different temperature than its external solution. This situation is more probable when the vesicles are intact, than when ions are cycling rapidly across the membrane, as in leaky vesicles.

The vesicles are prepared in a manner that keep their inside buffered. We can thus expect that there is also an interior buffer heat effect proportional to the proton flux. With a small change in the temperature of the interior vesicle, the measurable heat flux to the vesicle interior is:

$$J'^{,o}_q = C^o_p\frac{\mathrm{d}T^o}{\mathrm{d}t} + J_H\Delta H(\mathrm{buff}) \tag{22}$$

Here C^o_p is the heat capacity at constant pressure of the vesicle interior solution. A positive value of $J'^{,o}_q$ means that heat is transported from the membrane into the internal

[1] In an earlier article, Kjelstrup et al. (2005) related the calorimetric heat production, $-J^{\mathrm{cal}}_q$ erroneously to the system's entropy production.

Table 1 ATPase hydrolysis and heat release in leaky vesicles

Ca^{2+}-ATPase source	Reaction rate r (μmol/mg min)	Calorimeter J_q^{cal} (mJ/mg min)	J_q^{cal}/r (kJ/mol)
White muscle	6.00 ± 0.52 (3)	−280 ± 40	−47 ± 5
Red muscle	1.32 ± 0.30 (6)	−71 ± 20	−53 ± 5
BAT mito	0.68 ± 0.25 (7)	−38 ± 12	−60 ± 3

Data are taken from (de Meis 2003; Arruda et al. 2003). The number of experiments are given in parenthesis.The experiment was always started by adding the vesicle suspension to an assay medium. Assay media contained at start 1 mM ATP, 2 mM $MgCl_2$, 100 mM KCl, 10 mM Pi, 5 mM NaN_3, and 50 mM MOPS/Tris buffer to pH = 7.0. With SERCA from white and red muscle, 200 μM $CaCl_2$ and 200 μM EGTA. With SERCA from BAT mitochondria, 0.10 mM $CaCl_2$ and EGTA was used to give 1.6 μM free Ca^{2+}. There is no net Ca^{2+}-flux and the external and internal concentrations of Ca^{2+} and H^+ are the same

Table 2 Initial state ATPase activity and heat production following hydrolysis ($r > 0$) or synthesis ($r < 0$) in intact vesicles with a membrane gradient of Ca^{2+}

Ca^{2+}-ATPase source	J_q^{cal}/r (kJ/mol)	$J_H/r + \Delta_r N$	$J_q'^{o}/r$ (kJ/mol)	$C_p dT/rdt$ (kJ/mol)	$J_q'^{i}/r$ (kJ/mol)
White muscle	$-\frac{159}{1.65} = -96 \pm 8$	−1.64	−16	−45	−42
Red muscle	$-\frac{15}{0.25} = -60 \pm 7$	−1.48	15	−9	−11
BAT mito	$-\frac{38}{0.37} = -103 \pm 20$	−0.96	−35	−43	−61
White muscle	$\frac{1.8}{0.069} = -26 \pm 6$	−3.3	107	25	81

Table 1 gives conditions for the three first experiments, see de Meis (2002, 2003) and Arruda et al. (2003). In the synthesis experiment with white muscle SERCA, 0.1 mM ADP was used in combination with 4 mM $MgCl_2$ and 5 mM EDTA (de Meis 2002). The uncertainties in the original data lead to errors in the heat fluxes that are of the order ±20 kJ/mol

solution into the vesicle interior. A negative value for $J_q'^{o}$ means that the vesicle interior is getting colder.

We proceed below to interpret calorimetric experiments by de Meis and coworkers (de Meis 2001, 2002, 2003; Arruda et al. 2003), using Eqs. (15–22). The equations are general, in the sense that they apply whether ATP is hydrolyzed or synthesized. In the first case, r and J_{Ca} are positive; in the second case, these rates and the corresponding reaction enthalpies change their sign.

Heat production in SERCA isoforms

The Ca^{2+}-ATPase is found in various forms in all animal tissues (de Meis 2002). Three distinct genes encode three isoforms. The physiological meaning of the isoform diversity is not clear. The animal red muscle expresses SERCA 1 and SERCA 2a, while the white muscle expresses the SERCA 1 gene. SERCA 2b and SERCA 3 are expressed in non-muscular tissues such as blood platelets (Arruda et al. 2003).

It has been observed that the rate of ATP hydrolysis and the rate of heat released during ATP hydrolysis vary with the form of SERCA. The heat released is remarkably proportional to the rate of hydrolysis, as measured by isotope experiments. The ratio is constant within experimental uncertainties, if the SERCA vesicles are made leaky to Ca^{2+} by the drug calcimycin, A21187, see Table 1. A variation in the ratio has been found, depending on the SERCA isoform, when a gradient is allowed to build across the vesicle membrane, see Table 2. It is an aim of this analysis to interpret these experimental results.

Heat flows across leaky vesicles

De Meis (2002) and Arruda et al. (2003) determined the ATPase activity and the accompanying heat release in vesicles from white and red muscle tissue leaky for Ca^{2+}. De Meis (2003) studied also the same property of Ca^{2+}-ATPase from brown adipose tissue (BAT) mitochondria. Results were reported as $J_q^{cal}/r_{hydr} = \Delta H^{cal}$.

In leaky vesicles there is no net calcium flux ($J_{Ca} = 0$), and $r \approx r_{hydr}$. The enthalpy change of reaction (5) is small (enthalpies of mixing of dilute solutions are small, Honig 1995). In Eq. (17) this means that

$$\Delta H_{Ca/2H} \approx 0 \quad \Delta H_w \approx 0 \tag{23}$$

The rapid cycling of Ca^{2+}, makes it likely that $J_q'^{,int} \approx 0$. It is then likely that also $J_H = 0$. We shall see that this assumption is supported. For leaky vesicles we therefore apply:

$$\begin{aligned} J_q^{cal}/r &= \Delta H_{hydr} - (J_H/r + \Delta_r N)\Delta H(\text{buff}) \\ &= -51 \pm 4\,\text{kJ/mol} \end{aligned} \tag{24}$$

The results for J_q^{cal}/r for white and red muscle Ca^{2+}-ATPase are −47 and −53 kJ/mol in Table 1. The slightly different catalyst in the two cases should not have an impact on this result. Since the external solutions are the

same, we find it appropriate to use the weighted average for an interpretation of J_q^{cal}/r. With $\Delta_{\mathrm{hydr}}H = -26$ kJ/mol, $-[J_H/r + \Delta_r N]\Delta_r H(\mathrm{buff}) = -25$ kJ/mol. The value of $\Delta_r H(\mathrm{buff})^0$ for protonation of Tris is -47 kJ/mol, while for MOPS it is -22 kJ/mol (Bianconi 2003). The average value of these, -33 kJ/mol, gives $J_H/r + \Delta_r N = -0.75$, a value that is very close to $\Delta_r N_H = -0.76$ (Alberty 2003). De Meis et al. (2005) measured the average buffer enthalpy of the present mixture to -16 kJ/mol by titration with HCl. Their estimate for $\Delta_r N$ was -0.3. This gives a smaller correction to J_q^{cal}/r.

Taking the experimental results as a basis, the proton efflux in leaky vesicles in not negligible, while the data of Alberty (2003) and Bianconi (2003) indicates a negligible proton flux.

The experiments with Ca^{2+}-ATPase from BAT mitochondria gave -60 ± 4 kJ/mol (de Meis 2003) with a slightly different assay medium (see Table 1). The different assay compositions may explain the larger numerical value.

Heat flows across intact vesicles

Heat flows were also measured in *intact* vesicles during hydrolysis and synthesis (Arruda et al. 2003; de Meis 2003). A Ca^{2+} gradient was formed during the initial uptake. The uptake was mostly completed within 10 min while a heat production was recorded up to 30 min. Our analysis concerns the initial period. Constant values of r_{hydr} and J_q^{cal} were observed. In the initial phase, the reverse reactions are negligible, so $J_{\mathrm{Ca}}^{\mathrm{upt}} \approx J_{\mathrm{Ca}}$ and $r \approx r_{\mathrm{hydr}}$. Again we assume that $J_{\mathrm{Ca}}^{\mathrm{upt}}\Delta H_{\mathrm{Ca/2H}}$ is negligible compared to $r\Delta H_{\mathrm{hydr}}$, or validity of Eqs. (22). During the initial Ca^{2+}-uptake, Eq. (19) then gives:

$$J_q^{\mathrm{cal}}/r = J_q^{\prime,o}/r + \Delta H_{\mathrm{hydr}} - (J_H/r + \Delta_r N)\Delta H(\mathrm{buff}) \qquad (25)$$

A flux J_{Ca} proportional to r explains that $J_q^{\prime,o}/r$ is constant. The results for r_{hydr} and J_q^{cal} are given in Table 2. We calculated $J_q^{\prime,o}/r$ using the buffer values from Table 1, setting the proton flux equal to minus twice the Ca^{2+} flux. The value $\Delta_r N = -0.76$ was again used for hydrolysis.

We also assumed that the vesicle interior served as a thermal reservoir, thermally shielded from the exterior and the calorimeter. The ratios $J_q^{\prime,o}/r$ in column 5 applies to this situation. We next subtracted the buffer contribution. The cooling of the vesicle interior is listed in the last column. The results show, within the error of the experiments, that the membrane processes can lead to heat transport from the vesicle interior to the suspension during hydrolysis conditions. Under synthesis conditions, the opposite is true, heat is transferred to the interior.

Discussion

A first law analysis teaches us how energy is conserved. The second law gives the spontaneous direction of a process. Both laws must be obeyed. We show therefore first that the calculated processes are in agreement with the second law of thermodynamics, since this is an issue when heat is transported from a low to a high temperature. We continue to discuss the meaning of a negative heat flow to the vesicle interior, and the prediction that follows from this; that the Ca^{2+}-ATPase is also a heat pump.

Spontaneity requirements

The thermodynamic data must be consistent with the observed direction of spontaneous change. According to Nicholls (1982), $\Delta G_{\mathrm{hydr}} = -57$ kJ/mol for 298 K, pH = 7.0, 10 mM $MgCl_2$, Pi = 10 mM and ATP/ADP = 10^3. In the experiments where synthesis is observed (Table 2, line 4), the Pi concentration is again 10 mM, while ADP was 0.1 mM. The reaction Gibbs energy for the synthesis is around 25 kJ/mol with a ratio ATP/ADP of 10^{-3} (Nicholls 1982).

At constant pH, the Gibbs energy change for the exchange reaction (5) is:

$$\Delta G_{\mathrm{Ca/2H}} \approx -T\Delta S_{\mathrm{Ca/2H}} = RT\ln\,[\mathrm{Ca}^o]/[\mathrm{Ca}^i] \qquad (26)$$

At the start of the uptake experiment, there is no gradient in Ca^{2+} across the membrane, and $\Delta G_{\mathrm{Ca/2H}} = 0$. At stationary state the driving force is $-T\Delta S_{\mathrm{Ca/2H}}$. The entropy change corresponding to a concentration ratio of 10^4 (Møller et al. 1996; de Meis 2001) is -57 J/K mol. The entropy change is negative, since the exchange reaction is not spontaneous during Ca^{2+}-uptake. With the temperature of the experiment, 35°C, $\Delta G_{\mathrm{Ca/2H}} = 24$ kJ/mol.

At constant temperature and pressure, the spontaneity criterion of the second law is that the Gibbs energy changes are negative. This is likely with the numbers given above. The entropy production σ becomes, following Caplan and Essig (1983)

$$T\sigma = -r\Delta G_{\mathrm{hydr}} - J_{\mathrm{Ca}}\Delta G_{\mathrm{Ca/2H}} > 0 \qquad (27)$$

We can here neglect the contribution from the thermal force, see however Kjelstrup et al. (2005).

During uptake of Ca^{2+} the positive $\Delta G_{\mathrm{Ca/2H}}$ (24 kJ/mol) must be compensated by the reaction Gibbs energy for hydrolysis, $\Delta G_{\mathrm{hydr}} = -57$ kJ/mol. This is obeyed for all possible values of J_{Ca}/r. Likewise, the ion exchange reaction ($\Delta G_{2H/\mathrm{Ca}} = -24$ kJ/mol) can possibly drive synthesis, because $\Delta G_{\mathrm{synth}} \approx 24$ kJ/mol. Equation (27) is obeyed for synthesis because $|J_{\mathrm{Ca}}\Delta G_{2\mathrm{H/Ca}}| \approx |r\Delta G_{\mathrm{synth}}|$. The observed ratio J_{Ca}/r was near unity.

We conclude that the second law is obeyed in both cases.

Is the Ca^{2+}-ATPase also a heat pump?

In the analysis of data from Table 1, we showed that the observed heat production in the calorimeter was probably due to the hydrolysis reaction plus an accompanying shift in the buffer equilibrium. The data did not justify a *net* transmembrane flux of protons in this case, where the vesicles were leaky. The finding has support in other experiments on leaky vesicles. In the presence of agents that modify the transport properties like DMSO and NaF, and in thyroid treated tissue (de Meis 1997; Arruda et al. 2003), it was found that the heat production was not significantly affected.

In order to explain the calorimetric heat production in Table 2, we needed to first invoke proton transport across the membrane, to yield an increased buffer effect. This was not enough. In addition to that, a measurable heat flux across the vesicle membrane was likely. The amount of heat that the Ca^{2+}-ATPase was taking from the vesicle interior and was transporting to the suspension of vesicles varied. A high negative value was found for Ca^{2+}-ATPase from white muscle and from BAT mitochondria. When SERCA came from red blood cells, the heat flux was near zero.

In the first-mentioned cases, it appeared that the vesicle interior became cooler during hydrolysis, and warmer during synthesis. What is the origin of these processes, one may then ask? In the absence of net changes in the membrane itself, the changes must be related to the vesicle interior and exterior. And the process seems to be a reversible one. This means that the heat production must depend on the coupled ion exchange, $Ca^{2+}/2H^{+}$, and possible also on water transport. The assumptions (23) should be further examined, however, as it it known that changes in water activity is important for the functioning of the enzyme (de Meis et al. 1980; de Meis and Inesi 1982).

Is the vesicle interior cooling realistic?

Table 2 showed that the vesicle interior became cooler during hydrolysis; mostly in the case of Ca^{2+}-ATPase from white muscle and BAT mitochondria. Is a heat change of this order of magnitude realistic? In order to answer this, let ut estimate the temperature drop of the vesicle interior interior using the data in Table 2.

Reconstitution of Ca^{2+}-ATPase was reported by Dalton et al. (1999) to give vesicles around 100 nm in diameter, or with a volume of $5 \times 10^{-22} m^3$. The heat capacity of water is 4.2×10^6 J/K m^3, giving a heat capacity of the vesicle interior equal to 2×10^{-15} J/K. One Ca^{2+}-ATPase has the mass 1.8×10^{-16} mg. It can thus lead to a heat flux around 3.2×10^{-17} J/min according to Table 2, data for white muscle. We find then that the temperature of the vesicle interior can change by 0.2 K over 15 min by heat transport through one Ca^{2+}-ATPase. Five Ca^{2+}-ATPases per vesicle can give therefore a cooling of the order of magnitude of 1 K. This is possible, given the fluctuations in the system. A small temperature difference is rather difficult to thermostat by the calorimeter, as the vesicle membrane will be shielding the vesicle interior from the thermostat.

Reversible heat transport during ATP-hydrolysis

What is the reversible process that can generate a sensible heat effect in the vesicle interior during hydrolysis with intact vesicles? We have seen that the proton flux can play a role. Also the Ca^{2+}-flux and gradient must be important, as stated already by de Meis (2001, 2002) and Arruda et al. (2003).

When Ca^{2+} is transported from the low external concentration to the high vesicle interior concentration, there is an overall entropy change in the system that is negative (see the above subsection). Some entropy (heat) is needed on the vesicle interior side where Ca^{2+} appears, but more entropy (heat) is liberated on the external side where Ca^{2+} is removed. The amount is proportional to the logarithm of the concentration. The result is that heat is moved in the opposite direction of Ca^{2+}. Effects of proton translocation must be added. During uptake net heat is moved from the vesicle interior to the solution in which the vesicles are suspended. The calorimeter will therefore experience a larger heat production with intact vesicles during uptake than with leaky ones. During coupled efflux or synthesis, heat is moved in the other direction. In this sense, the Ca^{2+}-ATPase in the vesicle membrane can act as a heat pump.

When the Ca^{2+} gradient disappears and the concentrations on both sides are the same, as in leaky vesicles, the heat that is liberated in the solution in which the vesicles are suspended when Ca^{2+} is transported is the same as that removed from the vesicle interior. When the uptake of Ca^{2+} is zero in intact vesicles, one has reached a point with a balance of forces; the chemical force of the hydrolysis is balancing the osmotic force plus a possible thermal force.

In order to describe transport, one must write the fluxes and forces of the system, see Kjelstrup et al. (2005). Transport of heat by an ion flux gives a non-zero coupling coefficient between the heat and ion flux in non-equilibrium thermodynamics theory, see Kjelstrup (2005). Such a coupling means also that the heat flux is of a reversible type. It means that a temperature gradient can also be used to drive an ion flux, as has indeed been observed (Barata and de Meis 2002). Similarly, there is also possible a

reversible contribution to the heat flux from the chemical driving force. The fact that the heat flux changes direction under synthesis in Table 2, supports this view.

In particular the SERCA 1 Ca^{2+}-ATPase in white muscle is able to work as ion pumps, but clearly also as heat pumps because of their large ATPase activity. This may explain why SERCA 1 type Ca^{2+}-ATPases dominate in tissues where thermal regulation is important, while SERCA 2 type Ca^{2+}-ATPases dominate in red muscle and blood platelets where this heat flux is not so central (see Table 2 row three).

The coupled and uncoupled Ca-ATPase

The arguments so far have been based on net rates. Only net rates give effects on a thermodynamic level. It is not possible on this level to distinguish between a situation where an ion enters the vesicle interior and returns via some pathway, or the situation, where the ion "slips" and returns before it has entered. These situations can be distinguished using unidirectional rates, however.

The difference between SERCA 1 and 2 was related by de Meis and coworkers to their different unidirectional rates of hydrolysis, and Ca^{2+}-uptake abilities. The unidirectional Ca^{2+}-exchange at stationary state was used (Arruda et al. 2003) to determine these. While de Meis and coworkers argued that the state of zero uptake rate ($J_{Ca} = 0$) gives heat production beyond the enthalpy of hydrolysis, this is not possible from a thermodynamic point of view. A continuous movement of water and protons to the solution in which the vesicles are suspended takes most likely place, and should be investigated further. The results of Mall et al. (2006), that sarcolipin changes the heat production by reconstituted Ca^{2+}-ATPase from white muscle in intact vesicles, may be seen in the same context. The nature of the coupled versus uncoupled pump therefore still holds the key to a full explanation of the results.

Conclusion

Equations have been developed and used with calorimetric experiments on leaky and intact vesicles from SERCA. The energy balances for the separate parts of the experimental system have been formulated. When the standard value is used for the enthalpy of hydrolysis, and buffer effects are accounted for, a large heat effect is unaccounted for in the heat production by intact vesicles. A likely explanation for this is that electroneutral proton and calcium exchange across the membrane contribute to the local energy balances. Using this assumption, we show that the interior solution of the vesicles is some cases can be cooled during hydrolysis and uptake of Ca^{2+}, and that the heat flux is reversed during synthesis.

The thermodynamic data obtained are consistent with the second law of thermodynamics. We therefore propose that the Ca^{2+}-ATPase can work, not only as an ion pump, but also as a heat pump. The analysis is general and may apply to other P-type pumps. It serves as a background for a more detailed second law analysis that is now in progress.

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