

Energy Transduction at the Cell Plasmalemma: Coupling Through Membrane Associated Adenosine Triphosphate?

Key Words: cellular respiration, ion transport, cytosolic enzyme systems, energy transduction, glycolysis

Adenosine triphosphate (ATP) is known to be the primary high energy compound used to perform work in mammalian cells.¹ ATP and its hydrolytic products, adenosine diphosphate (ADP) and inorganic phosphate (P_i), also have been shown to modulate a variety of enzymes involved in intermediary metabolism.² Classical biochemistry has considered the metabolic pathways of the cytosol to be free in solution and thereby governed by three-dimensional diffusion kinetics in relation to their substrates and modulators. In recent years, a different picture has emerged, one in which many if not all of active cytosolic enzyme systems are organized within the cytosolic space.³ Using non-biological structures, organized enzyme systems have been shown to elicit advantageous alterations in the kinetic parameters of these pathways, possibly by removing their dependence on the bulk phase concentrations of integral intermediates. The concept of enzyme organization is already firmly in place in relation to the structuring of mitochondrial metabolism.⁴ On the other hand, the organization of metabolic pathways within the cytosol is less clear. In this respect, our laboratory has been particularly interested in an apparent coupling

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between the energy requirements of the plasma membrane localized Na-K ATPase and cytosolic glycolytic energy metabolism. At present, information concerning the spatial organization and functional characteristics of this coupled system is limited. In this Comment we will survey relevant evidence concerning the nature of the interaction between these energy conversion pathways at the level of the plasma membrane, and discuss the potential central role of ATP and its hydrolytic product ADP. Within this framework, the functional advantages conferred to the cell by such an energy transducing system localized at the plasma membrane will be examined.

PLASMALEMMA-ASSOCIATED ATP, AND ITS RELATION TO THE Na-PUMP AND GLYCOLYTIC ENERGY METABOLISM

Mature human red blood cells (rbc) are devoid of mitochondria, and carry out net synthesis of ATP through glycolysis alone. As in most cells, the major fraction of the enzymes responsible for glycolysis are found in the aqueous cytoplasm. However, specific glycolytic enzymes have been found to possess significant affinities for binding to the rbc plasma membrane.⁵ It was proposed that two of these membrane associated enzymes, glyceraldehyde phosphate dehydrogenase (GAPDH) and phosphoglycerate kinase (PGK) together were capable of producing ATP which was preferentially utilized by plasma membrane bound Na-K ATPase.⁶ Subsequent investigation lead to the hypothesis that a membrane associated pool of ATP was in close proximity to the Na-K ATPase, and this pool was utilized to fuel active Na-K transport.⁷ The relations between the purported ATP pool, the membrane associated glycolytic enzymes, and ion transport by the Na-K ATPase were examined in detail using inside out vesicles (IOV) from the human rbc.⁸ IOV concentrated Na⁺ into the intravesicular space in the presence of ATP. The substrates for GAPDH and PGK were also found to support Na⁺ transport by the Na-K ATPase in the absence of added ATP. IOV incubated with ATP in the presence of hexokinase and glucose did not transport Na⁺, since ATP was rapidly hydrolyzed by the hexokinase. However, even

in the presence of hexokinase-glucose, the substrates for GAPDH-PGK were found to stimulate Na⁺ transport. This finding strongly argues for a pool of ATP which is accessible to the Na-K ATPase but not readily accessible to enzymes in the bulk phase. The inability of hexokinase to degrade the membrane associated pool of ATP indicates that the rate of turnover of the ATP pool is faster than diffusion of ATP from the pool into the bulk phase. Conversely, diffusion of ATP into this pool should limit the rate of the Na-K ATPase under conditions where flux through the coupled glycolytic pathway is limiting (absence of glucose).

FUNCTIONAL INTERACTIONS BETWEEN THE GLYCOLYSIS AND THE Na-K ATPase

In vascular smooth muscle, a similar coupling between the energy requirements of the Na-K ATPase and energy production via glycolysis has been observed.⁹ On the other hand, mitochondrial oxidative phosphorylation was coordinated with tension generation by the muscle. An interesting aspect of metabolism in this tissue is that the glycolytic and oxidative components of intermediary metabolism can be altered independently by regulating the coupled energy conversion processes suggesting that these metabolic pathways are functionally compartmented.¹⁰ Glycogen was utilized as a primary substrate for mitochondrial respiration during tension development, whereas glucose uptake was primarily catabolized to lactate and released from the cell.^{11,12} Studies using radiolabeled glucose to follow carbohydrate utilization indicated that there was little if any mixing of substrate from these two sources.^{11,12} Thus, at least two independent pathways were necessary, one for the catabolism of glycogen to pyruvate for subsequent oxidation and another for the conversion of glucose to lactate. This latter pathway apparently was regulated by the turnover of the Na⁺ pump. Again, the inability of intermediates from the two sources to mix indicates that diffusion within the cell, particularly related to some membrane localized component, was limiting.

In order to gain insight into the potential advantages conferred to the cell by a spatially localized energy conversion process, we studied the relation between ion transport and the glycolytic cas-

cade within isolated cell lines in which both metabolism and transport could be measured simultaneously. In cultured cells incubated as a suspension under aerobic conditions, approximately 30% of the basal rate of ATP production is supplied through glycolysis.^{13,14} This substantial contribution of glycolysis to the net cellular ATP requirements is a common characteristic of cells grown in culture. Ouabain was used to specifically inhibit the activity of the Na-K ATPase. Measurements of the decrease in the rates of lactate production and oxygen consumption after the addition of ouabain was used to quantitate ATP utilization by the Na-K ATPase. Ouabain was found to decrease oxidative phosphorylation by approximately 20%, whereas, lactate production was inhibited by nearly 50%.^{13,14} Similar observations have been made using the renal cell line LLC-PK1.¹⁵ These findings indicate that glycolysis is more sensitive to changes in the activity of the Na-K ATPase than is oxidative phosphorylation, and therefore are consistent with the notion that ATP production via glycolysis is preferentially utilized by the Na-K ATPase.

We performed a detailed investigation of the kinetics of ion transport by the Na-K ATPase using rous transformed hamster astrocytes, ascites tumors, and a canine kidney derived cell line MDCK. The kinetic characteristics of the Na-K ATPase were investigated under conditions where ATP was produced solely through oxidative phosphorylation (glutamine or lactate as the only exogenous substrate) or from both oxidative phosphorylation and glycolysis (glutamine or lactate, and glucose). K⁺ depleted-Na⁺ loaded cells were used for these studies to control for effects of the intracellular concentration of Na⁺ on pump activity, and allow for the measurement of K⁺ uptake with a K⁺ electrode. K⁺ uptake was elicited by the addition of K⁺ to the initially K⁺ free medium in which the cells were suspended. Since the cells were K⁺ depleted and Na⁺ loaded (i.e., the Na-K ATPase presumably is limited only by the absence of extracellular K⁺), addition of K⁺ to the medium leads to rapid K⁺ uptake. The initial rate of K⁺ uptake into the cells is dependent on the medium concentration of K⁺ immediately following the readdition of K⁺, due to the inherent affinity of the Na-K ATPase for K⁺ ($K_m = 3.0$ mM).¹⁶ After addition of a saturating level of K⁺, the initial rate of K⁺ uptake was faster by cells in which both oxidative phosphorylation and glycolysis were

operative.^{15,16} In MDCK cells, K⁺ uptake was faster at all [K⁺]_o in cells incubated with glucose and glutamine compared to those incubated with glutamine alone. Eadie-Hofstee analysis of these data indicated that an increase (60%) in the V_{max} of K⁺ uptake was responsible for the higher rates of K⁺ uptake (Fig. 1).¹⁶ This elevated V_{max} was related to an increase in the turnover of available transporters and not the insertion of additional transport sites, since the number of pump sites measured with ouabain binding were equal under both substrate conditions.¹⁶

Alterations in the intracellular concentrations of ligands of the Na-K ATPase (ATP and Na⁺) could be responsible for the changes in pump activity. Since [Na⁺]_i was high (initial concentration approximately 60 mM) relative to the K_m of the Na-K ATPase for Na⁺ (4 mM), this ligand of the pump could not be limiting transport.¹⁶ The use of different substrates to alter uptake via the Na-K ATPase suggested that alterations in [ATP]_i may be involved. However, the alterations in K⁺ uptake were found to be independent of any substantial change in [ATP]_i. Moreover, the stoichiometry of the transport mechanism (K⁺ uptake/ATP production) was equal to 2 over all rates and under both substrate conditions, indicating that the turnover of the Na-K ATPase and intermediary metabolism are closely coordinated.¹⁶ Thus, alterations in the cytosolic [ATP]_i are not likely to be responsible for the higher rates of transport found in the presence of glycolysis. Since the number of transport sites was unaltered, these findings suggest that the available transport sites may in some way be limited by diffusion of ATP from the cytosol to the site of utilization.

The apparent coupling between the turnover of the Na-K ATPase and ATP production via glycolysis has now been observed in a variety of cell and tissue types (Table I).¹³⁻²¹ Furthermore, specific interactions between energy providing and localized work performing pathways may be a more general phenomenon. Other examples of pathway interactions which may be involved in membrane localized energy transduction have been observed (Table I). Evidence has been presented suggesting that a specific interaction exists between glycolysis and Ca⁺⁺ transport by the Ca⁺⁺ ATPase localized at the plasmalemma in vascular²² and stomach²³ smooth muscle, and potentially the sarcolemma in cardiac muscle.^{24,25} In addition, ATP dependent K⁺ channels found in pancreatic islet

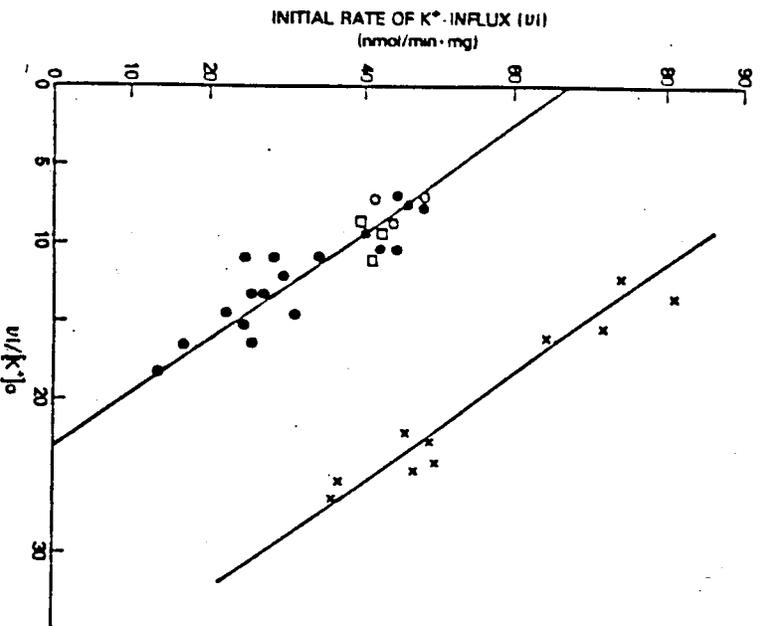


FIGURE 1 Eadie-Hofstee analysis of the initial rates of K^+ uptake in MDCK cells at varying extracellular K^+ concentration. Initial rates of K^+ uptake were measured after the addition of K^+ to cells which were previously K^+ depleted and incubated in nominally K^+ free media. The range of extracellular K^+ concentrations used to elicit K^+ uptake were from 0.9 to 5.6 mM. The rate of uptake was dependent on the concentration attained immediately following K^+ readdition. For more details see Ref. 27. The Y intercept is equivalent to the V_{max} of the transport mechanism, and the slope is equivalent to a $-K_m$ for K^+ . The lines are derived by linear regression. The lower regression line was calculated from uptake data obtained in the presence of glutamine as the only substrate. Experiments were carried out in the presence of various substrates: (\times) 10 mM glucose and 4 mM glutamine; (\circ) 4 mM glutamine; (\square) 10 mM 3-O-methyl glucose and 4 mM glutamine; (\bullet) 1 mM lactate and 4 mM glutamine. The slope and Y -intercept of the lower and upper regression lines are, -2.55 , 110.5 ($r = 0.94$) and -3.0 , 69.0 ($r = 0.86$), respectively. From Lynch and Balaban (Ref. 16), American Physiological Society (with permission).

TABLE I

Plasma membrane localized energy transduction coupled to glycolysis

Coupled Pathway	Cell or Tissue	Preparation	Reference
Na-K ATPase	Cardiac Muscle	Myocytes	17
		Isolated Hearts	18, 19
		Vascular	9, 10
	Smooth Muscle		
	Cell Culture	Intestinal A6, MDCK LLC-PK1 Ascites Tumor, HITcBII	20, 21 13 15 14
Ca ⁺⁺ ATPase	Smooth Muscle	Vascular	22
	Cardiac Muscle	Stomach Papillary	23 24
		Rat Heart	25
ATP Dependent K ⁺ Channels	Pancreas	Beta Islet Cells	26
	Cardiac Muscle	Myocytes	27

beta cells²⁶ and cardiac myocytes²⁷ may also preferentially derive energy through glycolysis or glucose utilization. Thus, a variety of localized energy transducing systems may exist. The physical nature and purpose of these specific interactions between energy providing and utilizing systems in terms of energetic advantage are not entirely clear.

The data presented to this point suggest that intracellular diffusion restrictions may lead to a direct coupling of ion transport and intermediary metabolism through a compartmentalized fraction of ATP and/or its hydrolytic products based on some form of enzyme structuring. However, it may be quite shortsighted to believe that the coupled energy transducing systems exist simply to minimize changes in the concentrations of these metabolites or assist in regulating energy metabolism, without a more direct function. To this end some very provocative information has recently

been presented which indicates that one of the primary glycolytic enzymes, glyceraldehyde phosphate dehydrogenase (GAPDH), may exhibit regulatory functions independent of its responsibilities in the glycolytic cascade. Purified muscle GAPDH has been found to autophosphorylate in the presence of ATP, adding the phosphate via an acyl bond.²⁸ This autophosphorylated form of GAPDH also has been found to have the capacity to transfer this phosphate to a group of proteins associated with a membrane microsomal fraction isolated from rabbit skeletal muscle. It is of interest that the activated forms of both the Ca^{++} ATPase and Na-K ATPase are known to be acyl phosphorylated intermediates.^{29,30} Although highly speculative, these observations imply functions for GAPDH other than those directly associated with its role in glycolysis. Thus, the spatial relation between GAPDH, and the other enzymes of the glycolytic cascade, with a transport ATPase may not only confer advantages in terms of the kinetics and regulation of metabolism, but also act by directly modulating the activity of the energy conversion process as well. Such a postulate is consistent with the findings of increased V_{max} of the Na-K ATPase in association with an activated glycolytic pathway in the MDCK cell line. However, without knowledge of the molecular organization of the coupled enzyme system, or quantitative evidence for the in situ kinetics of the system as a whole, it is difficult to evaluate if such a direct modification of enzyme activity is possible.

In the absence of a direct regulatory action, the most likely explanation for the observed coupling between these energy producing and utilizing pathways appears to be dependent on some form of diffusion limitation. The red cell data suggest that a plasma membrane associated pool of ATP may in fact be necessary to overcome diffusion limitations on ATP to the hydrolytic site of the Na-K ATPase.⁸ It is also noteworthy that a plasmalemma associated pool of ATP has been proposed based on studies of the electrical stability of the sarcolemma of ischemic rat hearts.³¹

ATP DIFFUSION IN A HOMOGENEOUS CYTOPLASM

The ATP dependence of enzymes localized at different subcellular sites within isolated hepatocytes has been examined.³² ATP sul-

furylase activity was used to estimate ATP in the aqueous cytoplasm, and Na-K ATPase activity was analyzed to determine ATP availability at the plasma membrane. The activity of the sulfurylase was directly proportional to the cytosolic ATP content under a variety of interventions which altered cell ATP, whereas Na-K ATPase activity was negligible after total cell ATP decreased to 40% of control. Therefore, these studies suggest that ATP gradients may exist between the intracellularly located sulfurylase and the plasmalemma, under highly stressed conditions. Another example of potential cell ATP gradients has been found in the renal proximal tubule.³³ A linear relation between Na-K ATPase activity and the intracellular ATP concentration was observed when ATP was varied (0.8–3.5 mM) by additions of rotenone, a mitochondrial uncoupling agent. Since the K_m of the isolated Na-K ATPase is approximately 0.4 mM, the linear relation between intracellular ATP and pump activity also indicates that ATP gradients may exist in the proximal cell. Morphologically, the mitochondria of renal proximal cells are closely associated with the basolateral membrane which contains the majority of the cells complement of Na-K ATPase molecules. The purpose for this close association may be the requirement to overcome gradients for ATP or its hydrolytic products. Although both the MDCK K^+ uptake data and the studies using metabolic inhibitors are suggestive of cell ATP gradients, mathematical analysis of the subcellular distribution of ATP based on experimentally derived parameters demonstrate that substantial gradients are unlikely to occur under physiological conditions.^{32,34}

Intuitively it is difficult to imagine gradients for ATP across cells of small diameter ($<10 \mu\text{m}$) with relatively high ATP concentrations (1–10 mM). We have illustrated this in a model of the MDCK cell system used to evaluate the concentration profile of ATP between the plasma membrane and a source of ATP in the cytosol, under conditions similar to those attained during the K^+ uptake experiments.²⁷ The model consists of a spherical cell with mitochondria distributed in its interior, and Na-K pumps distributed about the periphery (outer 1% of the cell volume) with dephosphorylation of ATP occurring in both the cytosol and at the plasma membrane (Fig. 2). ATP is produced only at the level of the mitochondria. The lowest rate of ATP utilization at the plasma membrane was set to approximate ATP utilization by the Na-K

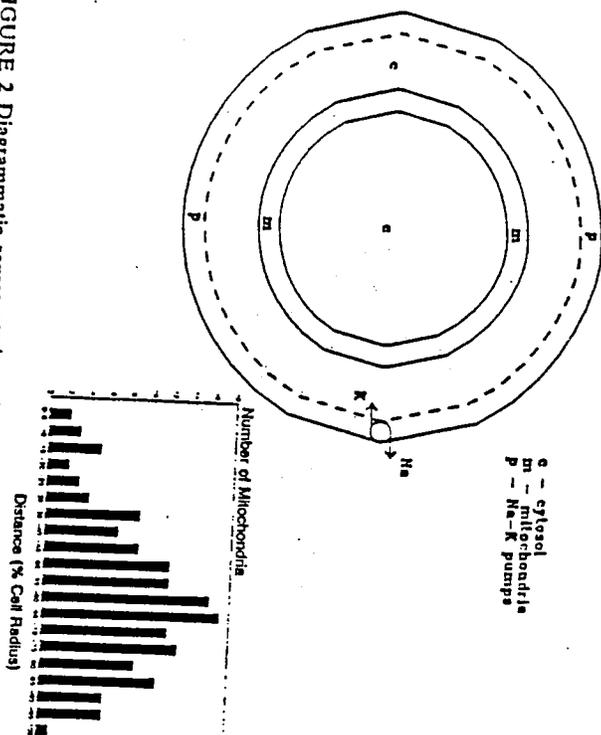


FIGURE 2 Diagrammatic representation of the model used for analysis of the distributions of ATP and its hydrolytic products ADP and P_i . The model consists of a spherical cell with mitochondria in its interior and Na-K pumps at its periphery with dephosphorylation of ATP occurring at a fixed rate in the cytosol and a variable rate at the plasma membrane. In MDCK cells, mitochondria occupy less than 10% of the cell volume with the most mitochondria located between 55–65% from the cell center (see inset). Therefore, calculations taking into account the restriction of mitochondria utilize this observed location. The Na-K ATPase was restricted to 1% of the radial distance, and therefore is enlarged by a factor of 10 in the diagram, for illustrative purposes. For a detailed description of the mathematical model, see Note. All values listed in Table II were taken from data measured from the MDCK cell population (Refs. 13 and 16) or are established values found in the literature. The inset diagram depicts a serial analysis of MDCK cell electronmicrographs, to estimate the volume of the cell occupied by mitochondria, as well as their approximate distribution within these cells. 150 mitochondria were observed from 9 micrographs of MDCK cells.

ATPase under conditions where extracellular K^+ was negligible, i.e., where pump turnover is low but not zero. This pump rate is designated as A_p in the model. The cytosolic ATP utilization rate was estimated from measurements of ATP production in MDCK cells after treatment with ouabain, i.e., in the absence of any ATP utilization by the Na-K ATPase. This value was maintained con-

stant. Elevations in pump rate were generated experimentally by the addition of K^+ to the medium in which the cells were suspended. For details of the experiment and the model simulation see the legend of Figs. 2 and 3, and Ref. 16.

At any radial position in the cell, the change in $[ATP]$, $[ADP]$, and $[P_i]$ with respect to time is equal to the diffusive flux of each species plus a reactive term that consists of the production minus the consumption of the species. The individual species were related through their chemical equilibrium. (See Note at the end of this article for complete derivation of the model.) Solutions of this model using the values shown in Table II indicate that neither ATP or ADP are diffusion limited between the plasma membrane and cytoplasm when the species diffusion constants are those measured in pure water (10^{-5} cm²/s, profiles not shown). When the ATP production source is uniformly distributed, and species diffusivities are those estimated for the cytosolic environment (10^{-6} cm²/s),^{34,35}

TABLE II
Parameters and experimentally derived values

Symbol	Value	Description
r_c	6.4×10^{-4}	Cell radius (cm) ^a
r_0	b	Inside radius for distribution of mitochondria (cm)
r_1	b	Outside radius for distribution of mitochondria (cm)
r_2	$r_c - 1\% r_c$	Inside radius for distribution of pumps (cm)
C_0^i	4.0×10^{-3}	Initial $[ATP]$ (M) ^a
C_0^j	28–40	Initial $[ADP]$ (μ M) ^a
C_0^k	1.0×10^{-3}	Initial $[P_i]$ (M) ^a
K_{eq}	$C_0^j C_0^k / C_0^i$	Apparent equilibrium constant (M)
k_p	1.0×10^{-3}	Dephosphorylation rate at equilibrium (s ⁻¹)
D_1	$10^{-4} - 10^{-3}$	ATP diffusion (cm ² /s) ^b
D_2	$10^{-4} - 10^{-3}$	ADP diffusion (cm ² /s) ^b
D_3	$10^{-4} - 10^{-3}$	P_i diffusion (cm ² /s) ^b
A_m	7.8×10^{-14}	Initial rate of phosphorylation at mitochondria (moles/min)
A_c	7.0×10^{-14}	ATP consumption rate in cytosol (moles/min) ^c
A_p	7.8×10^{-15}	Initial rate of ATP consumption by Na/K ATPase at plasma membrane (moles/min) ^c

^aValue measured for MDCK cells; see Refs. 13 and 16.
^bSee text for value.
^cSteady state value at $A_c = 0$, $A_p = 7.8 \times 10^{-15}$.
^dEstimated value; see Refs. 34 and 36.

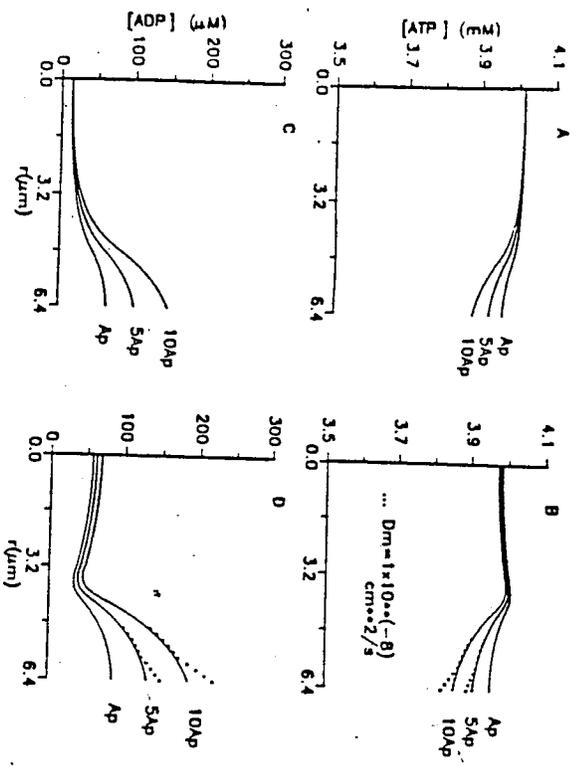


FIGURE 3 Simulated concentration profiles of ATP and ADP at three different ATP turnover rates. On the ordinate are the calculated steady state concentrations. The abscissa represents the position in the cell with 0 indicating the cell center and 6.4 the cell surface. All curves are generated under conditions where cell metabolism and ion transport are at a steady state. Differences in the centerline concentrations of adenine nucleotides after transitions A_1 are due to the differences in ATP utilization (and ADP production) associated with the increased turnover of the Na-K ATPase. Since the species are related through their chemical equilibrium, new steady state set points are attained after alterations in turnover. The membrane associated ATP utilization rate under low transporting conditions (A_1) is estimated from the ouabain sensitive components of energy metabolism measured from MDCK cells incubated without external K^+ (Ref. 13). Transitions in A_1 were thereby stimulating transport. $5A_1$ is a typical rate of transport measured experimentally. $10A_1$ is the calculated V_{max} of the Na-K ATPase in the MDCK cell preparation; this rate of utilization was never attained experimentally (Ref. 16). (A and C) Concentration profiles when the ATP production source (mitochondria) are uniformly distributed over the initial 75% of the cell interior. (B and D) Concentration profiles when the mitochondria are restricted to 10% of the cell interior at a location 55–65% from the cell center. Species diffusivities are homogeneous across the cell, and are equal to 10^{-6} cm^2/s unless otherwise noted. Dotted lines (...) indicate experiments where the diffusion coefficient was decreased by two orders of magnitude (i.e., 10^{-8} cm^2/s) in the plasma membrane compartment only.

significant gradients for ADP, but not ATP or P_i are generated after elevations in pump rate (Figs. 3A and 3C). Similar profiles are generated for P_i as are found for ADP, under all conditions (not shown). However, due to the high initial P_i concentration (1 mM), relative to that of ADP (28 μM), no significant gradients for this metabolite are observed.

It also has been suggested that mitochondria are clustered at specific subcellular locations, rather than distributed uniformly throughout the cytoplasm.³² Serial analysis of electron micrographs of MDCK cells indicate that mitochondria occupy less than 10% of the total cell volume, and the profile of their distribution suggests that mitochondria are more likely to reside near the outer 2/3 of the cell with most found within 55–65% of the distance from the center (Fig. 2 inset). Figures 3B and 3D show the concentration profiles for ATP and ADP when mitochondria are restricted to 10% of the radial line at a location which covers a distance of 55–65% from the cell center. These solutions show once again that ATP is not diffusion limited between the restricted mitochondria and the plasma membrane, although small gradients (<5%) may exist after large elevations in pump turnover ($10 \times A_1$). On the other hand, localization of the ATP production source at 55–65% of the cell radius results in a minimum [ADP] at the mitochondria, a gradual increase toward the cell center (due to ATP consumption in the cytosol) and a steep increase to a maximum [ADP] at the cell plasma membrane due to enzyme activity at the pump sites. These gradients may be particularly steep (three-fold increase over 2.9 μm) under conditions where ion transport is dramatically elevated, as found after readdition of K^+ to K^+ depleted cells. Furthermore, a significant ADP gradient (1.5-fold increase at the plasma membrane) was generated under low turnover conditions (at A_1). The magnitude of the gradient is due to the low free cellular ADP concentration relative to that of ATP and P_i . Similar conclusions have been drawn for myocardial cells and skeletal muscle.^{34,36} Those observations provide the basis for the hypothesis that phosphocreatine-creatine act as a shuttle or buffering mechanism for ADP between the myofibrils and mitochondria through the localization of creatine kinase at these two subcellular sites.^{34,35} An uncertainty in the model is the actual diffusivity of the phosphate compounds within the cell cytosol. One theory of cell water

structure considers the major fraction of cell water to exhibit properties similar to those of free water.³ In general, most species with molecular weights or hydration radii similar to ATP exhibit diffusivities which are 2 to 10 times less than the values measured in pure water.^{34,37} These lower rates of diffusion are thought to be due to transient interactions of the small charged molecules with cellular ultrastructural elements. On the other hand, water in the vicinity of cellular membranes (vicinal water) is thought to exhibit properties different from the bulk phase due to layering effects. In order to simulate this situation, as well as account for the potential effects of enzyme structuring on the development of metabolite pools at the plasma membrane, we varied the respective diffusivities of the species in the outer 1% of the cell volume. That is, the diffusion coefficient over 99% of the cell was held constant at 10^{-6} cm²/s, and the diffusion coefficient in the plasma membrane compartment was reduced by 2 orders of magnitude (Figs. 3B and 3D). Of particular interest is the finding that this 100-fold decrease in the diffusivity of ATP in the membrane compartment does not lead to a significant gradient for ATP between the pump sites and the cell interior. Thus, an ATP gradient between the mitochondria and the plasmalemma is not likely to account for the lower rates of uptake in the absence of glycolysis, even in the presence of a significant barrier to ATP diffusion at the plasmalemma. For any true pool of ATP or ADP to be sequestered at the plasma membrane, a barrier diffusion greater than 100-fold would be necessary.

NATURE OF THE ATP COMPARTMENT

The absence of ATP diffusion gradients even under highly active conditions suggests that a specific pool of ATP at the plasmalemma is not necessary to remove energy limitations *per se*. Yet the functional uptake data indicate that the coupling of ion transport with glycolytic ATP production at the plasma membrane confers distinct advantages with respect to the attainment of maximal transport rates. The physical nature of this interaction is vague. Some form of ATP compartment at the plasmalemma, generated by the presence of glycolytic enzymes in juxtaposition to the ATP binding site of the Na-K ATPase, is suggested by the red blood cell data.^{7,8}

However, the diffusion model indicates that this interaction would have to confer a barrier to the diffusion of ATP greater than 100-fold in order to significantly limit bulk cellular ATP from diffusing to the utilization site, or conversely glycolytically produced ATP from diffusing out of the compartment. There is no evidence in support of physical barriers to the diffusion of small charged molecules like Na⁺, K⁺ or ATP, which would be associated with this compartment.^{35,37} Therefore, it is unlikely that the coupling of pathways is dependent on or elicits compartmentalized metabolite "pools." An alternative to a compartmentalized "pool" of a metabolite such as ATP is the concept that the spatial organization, and subsequent coordinated action of the enzymes involved in the coupled system limits mixing with the bulk phase. According to this hypothesis, intermediate metabolites are passed or "tunneled" from one enzyme of the pathway to the next, thereby avoiding three-dimensional diffusion kinetics.^{38,39} The intermediates are thought to be essentially bound until they diffuse from the system as end products. Therefore, the diffusion limitation is due to the chemical reaction occurring faster than diffusion from the active site. The potential advantages of metabolite tunneling within coupled enzyme systems in terms of increases in the kinetic "efficiency" of metabolic pathways has been described theoretically^{40,41} and demonstrated with matrix bound metabolic sequences.^{42,43} However, no theoretical model has been developed which considers the advantages conferred by enzyme coupling between ATP synthesizing and utilizing processes in biological systems. The K⁺ uptake experiments in MDCK cells, demonstrating an increase in V_{max} of transport by the Na-K ATPase in the presence of an active glycolytic pathway, implies that the advantages conferred to metabolism by membrane organization of enzyme pathways may be extended to the localized energy transduction processes. Once again, more knowledge of the physical basis and spatial organization of these interactions is necessary before a more quantitative approach can be taken.

Another implication of the modeling experiment is that mitochondrial clustering may contribute to the development of significant ADP concentration gradients under both low work conditions and during work transitions, and therefore, compartmentation of energy metabolism may provide advantages in regulatory control.

by limiting these gradients. In this respect, the glycolytic enzymes associated with the plasmalemma may act as an ADP "buffer," eliminating potentially high localized ADP concentrations. Recent studies carried out using a Na-K ATPase preparation reconstituted into lipid vesicles provides evidence that near physiological concentrations of ADP may inhibit the activity of the Na-K ATPase. A non-competitive inhibition of the Na-K ATPase was observed with a K_i of approximately 100 μM .⁴⁴ This evidence is consistent with the notion that buffering of local ADP concentration may be important during rapid transitions in pump turnover and subsequent ADP production, as elicited in the K^+ uptake experiments. However, another investigation of the effects of ADP on Na-K ATPase activity in cardiac sarcolemmal vesicles indicates that ADP acts as a competitive inhibitor of a low affinity ATP binding site.⁴⁵ This is consistent with studies performed using erythrocyte ghosts in which ADP was found to be a weak competitive inhibitor of Na-K ATPase turnover.⁴⁶ It has been suggested that the discrepancies between these studies are attributable to differences in experimental conditions and/or the source of the preparation. Thus, any direct kinetic effect of localized elevations in ADP on the Na-K ATPase remains an open question.

SUMMARY

The functional association of specific energy conversion and work producing pathways for the purpose of localized subcellular energy transduction may be an important feature of cellular function. Increases in the "kinetic efficiency" of energy conversion and coordinated metabolic regulation are potential advantages of such subcellular organization. As previously noted, the apparent subcellular organization on which these coupled systems are based appears to be dynamic, and the advantages are likely to be attributable to metabolite tunneling within the organized system rather than through static isolation via diffusional barriers.⁴⁷ Another possible feature of these systems is the potential for direct modulation of activity through coordinated modification of the energy conversion process. However, without a more detailed understanding of the molecular organization of such systems, it is presently difficult to quantitate their properties. To this end, a variety of

biochemical and physical techniques are being utilized to probe the molecular nature of submembrane organization and function.

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Note

The model is described by a system of partial differential equations of the form

$$\frac{\partial C}{\partial t} = \nabla^2 (D \cdot C) + S \tag{1}$$

where $C = [C_1, C_2, C_3]$ is the vector of concentrations of ATP, ADP and P_i ; t is time; $D = [D_1, D_2, D_3]$ is a vector of diffusion constants; and the source vector, $S = [S_1, S_2, S_3]$, describes the production and consumption of species by chemical reaction. Assuming spherical symmetry, the chemical sources depend on radial distance, r , and are defined by the following equations:

$$S_i(r) = \frac{k_{-i}}{K_{eq}} \cdot C_2 \cdot C_3 - k_{+i} \cdot C_1 \quad \text{for } 0 \leq r \leq r_c \text{ (entire cell)}$$

$$+ \frac{a_m \cdot k_{+i}}{K_{eq}} \cdot C_2 \cdot C_3 \quad \text{for } r_0 \leq r \leq r_1 \text{ (mitochondria)}$$

$$- a_c \cdot k_{+i} \cdot C_1 \quad \text{for } 0 \leq r \leq r_2 \text{ (cytosol)}$$

$$- a_p \cdot k_{+i} \cdot C_1 \quad \text{for } r_2 \leq r \leq r_c \text{ (pumps)}$$

and

$$S_2 = S_3 = -S_1$$

where r_c is the cell radius; $r_i - r_o$ is the width of the annulus within which mitochondria are assumed to be distributed; and r_2 is the radius outside which the pumps are assumed to be distributed. K_{eq} is the apparent equilibrium constant, and k_+ is the rate of dephosphorylation of ATP at equilibrium. The rate of ATP phosphorylation in the mitochondrial compartment is

$$A_m = \frac{4}{3} \cdot \pi \cdot (r_1^3 - r_0^3) \cdot \frac{a_m \cdot k_+}{K_{eq}} \cdot C_2 \cdot C_3$$

It should be noted that this term does not saturate with ADP concentration. That is, no mitochondrial K_m term for ADP has been included. Instead, ATP production simply keeps up with consumption at all ADP concentrations. ATP consumption in the cytosol (A_p) and at the plasma membrane (A_b) are given as follows:

$$A_b = \frac{4}{3} \cdot \pi \cdot r_1^2 \cdot a_b \cdot k_+ \cdot C_1$$

$$A_p = \frac{4}{3} \cdot \pi \cdot (r_2^3 - r_1^3) \cdot a_p \cdot k_+ \cdot C_1$$

with constants a_m , a_b and a_p chosen so that $A_m = A_b + A_p$ at a steady state.

Equation (1) is solved numerically using a finite elements spatial discretization and a fully implicit approximation for the time derivatives [WODEPPER, IMSL]. We apply no flux boundary conditions.

$$\frac{\partial C_i}{\partial r} = 0 \quad \text{at } r = 0 \quad \text{and } r = r_c$$

and initial conditions

$$C_i(r, 0) = C_i^0 \quad \forall k$$

where the C_i^0 are specified.

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Polarized Light Scattering

Key Words: polarized light scattering, phase differential scattering, Mueller matrix, bacterial detection

INTRODUCTION

Elastic light scattering [1] has been used for over one hundred years to analyze aerosols, liquids, colloids, polymers, and interstellar dust.¹⁻⁶ It is a particularly attractive tool for biological studies. Fine structure can be probed remotely and non-invasively. Light scattering rarely interferes with biological structure or function.^{4,7} This is not the case with fluorescent dyes, stains, or certain kinds of microscopy.

In the past, the *intensity* (irradiance) of the scattered light was of prime concern. The *polarization properties* of the scattered light were often ignored. As a result, a considerable amount of structural information about the scatterer was overlooked.² In recent years, awareness of this fact has led to an increased interest in polarized light scattering.^{3,8-26}

This Comment briefly outlines the theory of polarized light scattering. Several techniques for polarized light scattering are then discussed, with special emphasis on experimental considerations and problems. One of these techniques—phase differential scattering—is particularly interesting because of its simplicity and relative freedom from experimental artifacts. Finally, the notes [1-11] appearing after the text are intended for readers interested in the practical aspects of polarization measurements.

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