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ATP / ATPase and Flux Activities in Human Red Blood Cells

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Abstract

ATP synthase [E.C.3.6.1.34] of 16 subunits and of Cu_A Cu_B prosthetic groups is also known as 'coupling ATPase', H^+-ATPase (Hajjawi, 2011; 2012a). This enzyme can utilize the energy released by the movement of protons down $\Delta\bar{\mu}_{H^+}$ to energize the synthesis of ATP. The enzyme consists of three subunits, an α subunit (112.5kDa) that couples ATP hydrolysis and ion transport, β subunit (45 kDa) and a small proteolipid γ subunit (10kDa) that copurifies with the protein but does not appear to be required for ATPase activity (Hardwicke and Fretag, 1981; Grüber, 2001). The α, β and γ subunits are in a ratio of 1:1:1. ATP has several known functions in the red blood cell (RBC). It fuels the Na^+ pump (Sachs, 2003; Gusev and Ivanova, 2004), which is thought to be involved in regulation of cell volume (Huber et al., 2001; Brugnara, 2003), and the Ca^{2+} pump (Romero and Romero, 2003), without which the cells take up Ca and subsequently shrink because of activation by Ca^{2+} of a K^+ channel in the membrane (Gibson and Ellory, 2003). In these cases, transfer of energy from ATP hydrolysis to the actively transported ions appears to involve specific ATPases in the membrane. ATP is also required for maintenance of normal cell structure; ATP depletion causes changes in the morphology (Gibson et al., 2000; Hajjawi, 1012b) and in the rheological properties (Henkelman et al., 2010) of the RBCs. These changes have clinical significance since RBCs low in ATP have poor post-transfusion viability (Yoshida and Shevkoplyas, 2010).Thus, RBC ATP and ATPase activities play an important role in research on the mechanisms of active transport and on the integrity of cell structure.

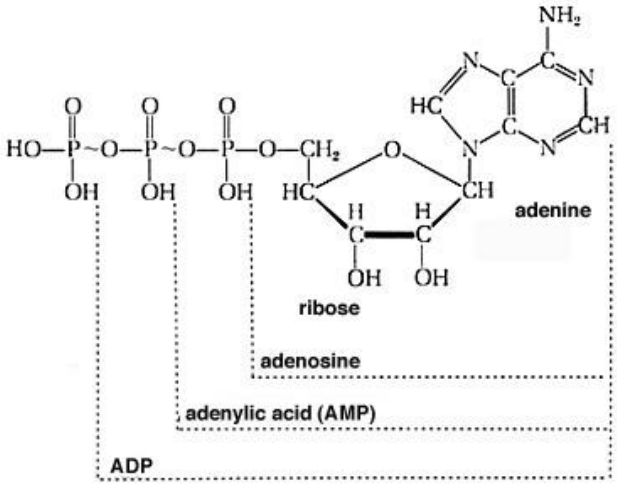
Keywords: Adenine-5'- triphosphate; erythrocyte; Na^+/K^+- ATPase; Ca^{2+} -ATPase; Mg^{2+}-ATPase; membrane cation pumps; coupling, transport; Gibbs energy; nicotinamide adenine dinucleotide phosphate –NADPH.

Introduction

A common feature of the various biological energy conservation systems is their ability to conserve energy in the formation of adenosine-5'-triphosphate (ATP). Fiske and Subbarow (1929) and Lohmann (1929) were the first to discover ATP independently from extracts of mammalian skeletal muscle and its structure (Fig.1) was established several years later (Maruyama, 1991). At pH 7, three of the four hydroxyl groups in the triphosphate side chain are fully dissociated (pK_1 = 2.1) and the fourth is predominantly so (pK_2 = 6.8). Thus under physiological conditions, the molecule is strongly negatively charged (ATP^{4-}), and it readily binds divalent cations, e.g. Mg^{2+} , Ca^{2+} (Lew et al., 2003;Muzyarnba et al., 2006).

Unfortunately, the chemical properties of ATP have been the subject of many misconceptions, particularly by biochemists (Oliveira, 2003; Scott, 2005). The idea that ATP is a 'high energy molecule' or that it contains a 'high energy phosphate bond' (Lipman, 1941), often denoted by the symbol ~, is incorrect.

Figure 1: The chemical structure of ATP.
ATP consists of the purine base adenine, the sugar ribose, and the three phosphate groups.ADP has two phosphate groups, and AMP has one phosphate group. The high-energy bonds between the phosphate groups are designed by wavy lines.



These misconceptions arise from the fact that when ATP undergoes hydrolysis at pH 7, particularly under the action of the enzyme

$$ATP^{4-} + H_2O \rightleftarrows ADP^{3-} + HPO_4^{2-} + H^+ \quad (1)$$

adenine triphosphatase (ATPase), the apparent equilibrium constant ($K^{'}$) of this reaction (Eq.1) is fairly high since it is favoured by the relatively high pH , and by the fact that the major products of ADP^{3-} and HPO_4^{2-} are both negatively charged resonance hybrids, i.e. they contain less free (Gibbs) energy than expected. At equilibrium,

$$\Delta G^{o'} = -2.303\ RT \log K^{'} \quad (2)$$

where $\Delta G^{o'}$ is the standard free energy of hydrolysis (kJ mol^{-1}), R is the gas constant (8.314 $jmol^{-1}$ K^{-1}) and T is the absolute temperature (K). Thus, at 298 K (25^0 C) an observed $K^{'}$ of 3.5 x 10^5 is equivalent to a $\Delta G^{o'}$ for ATP of – 31 kJ mol^{-1} (≡-7.4 kcal mol^{-1}) ; the minus sign (negative) indicates that the reaction will proceed spontaneously from left to right under these conditions and that the energy will be released from the system. This value is only moderately high and it is of course pH-dependent since a proton is one of the products. Therefore, the energy which is released during ATP hydrolysis reflects the nature of the products and the overall chemical structure of ATP molecule rather than the chemistry of the terminal phosphate bond itself. This is also true for ADP, where hydrolysis to AMP^{2-} and HPO_4^{2-} is again associated with a moderately high free energy change ($\Delta G^{o'}$ = -31 kJ mol^{-1}). The symbol (~) thus denotes that the only hydrolysis of a molecule at that point will lead to the release of a moderate to high amount of free energy. In practice, the simple hydrolysis of ATP to ADP and $P_{inorganic\ phosphate}$ with the release of heat occurs very rarely and is usually detrimental to metabolism when it does so (Laidler, 1987; Perrot, 1998).

Since the hydrolysis of ATP is an energy releasing (*exergonic*) reaction, then the synthesis of ATP from ADP and P_i requires a similar amount of energy, i.e. the reaction is *endergonic* to the extent of +31 kJ mol^{-1} under standard conditions. The harnessing of the free energy which is released during biological coupling of oxidation-reduction 'chemiosmetic' metabolic processes to the synthesis of ATP is

$$C_6H_{12}O_6 + 6O_2 + 38ADP + 38P_i \rightarrow 6CO_2 + 6H_2O + 38ATP \qquad \text{(Eq. 3)}$$

$$\Delta G^{o\,\prime} = -1860 \text{ kJ (445 kcal)} \qquad (3)$$

thus a convenient means of energy conservation (Mishra and Passow, 1969; Dudzinska et al., 2010). Unfortunately, only in the case of substrate-level phosphorylation is it also a relatively simple process. The energy needed for active transport makes a high demand upon the metabolic capacity of RBC (Eq. 3). As do most cells in the body, RBCs anaerobically catabolyze glucose to lactic acid via the Embden-Myerhof, or glycolytic, pathway **(**Pilkis and Claus, 1991**).** Since RBCs do not store glycogen, they must constantly catabolyze glucose from the bloodstream via this pathway and the hexose monophosphate shunt as a source of energy. The Embden-Myerhof pathway serves three functions in the RBC: ATP production, 2, 3- diphosphoglycerate (2, 3 DPG) production, and NADH production. For ATP production, the glycolytic pathway produces the RBC's major fuel source, ATP (glucose + 2 ADP + 2 Pi ; 2 lactic acid + 2 ATP). While several enzymes depend on ATP, glyceraldehyde-3-phosphate dehydrogenase and phosphoglycerate kinase particular are critical to RBC function: the Na^+-K^+ pump (Mercer and Dunham, 1981). The RBC's volume is maintained in large part by the Na^+-K^+ ATPase in its plasma membrane, which extrudes Na^+ from the cell together with osmotically obligated water molecules (Dieze- Silva et al., 2010).

We assign every element in its standard state a Gibbs energy of zero. The standard Gibbs energy of formation of any compound is then simply the Gibbs energy change, $_{\text{formation}}$, which accompanies the formation of the element in its standard state from its elements in their standard states. Then we can calculate the standard Gibbs energy change for any reaction, ΔG^o, by adding the Gibbs energies of formation of all of the products and subtracting the sum of the Gibbs energies of formation of all reactants:

$$\Delta G^o = \Sigma \Delta G^o_{\text{formation}} \text{ (products)} - \Sigma \Delta G^o_{\text{formation}} \text{ (reactants)} \qquad (4)$$

In order to maintain Na^+ and K^+ gradients at the surface of RBC, ions must be moved in a thermodynamically unfavourable direction ('uphill'):

$$\Delta G^o = RT \ln \frac{C_{1\,(\text{high})}}{C_{2\,(\text{low})}} \qquad (5)$$

ΔG^o is required to transport 1 mol permeant from concentration C_2 (low) → C_1 (high), {activity = activity coefficient (γ) x [concentration]}. For ionized permeant:

$$\Delta G^o = RT \ln \frac{C_{1\,(\text{high})}}{C_{2\,(\text{low})}} + zF\Delta\Psi \qquad (6)$$

where z is the valance of the ion, *F* is the Faraday and $\Delta\Psi$ is the potential difference across the membrane.

Hence, this uphill transport consumes energy of:

$$\Delta G_{\text{product}} = \Delta G^{\prime\,o} + RT \ln \frac{[ADP]\,[P_i]}{[ATP]} \qquad (7)$$

= - 52 kJ/mol (`12.43 kcal/mol)

where [ATP] = 2.25 mM, [ADP] = 0.25 mM, [AMP] = 0.02 mM and [P_i] = 1.65 mMin human RBC (Nelson and Cox, 2008, p.502). The amount of energy being determined by the concentration gradient of unionized permeants (Eq. 5), or electrochemical potential for ions (Eq. 6).

Although membrane proteins constitute more than 20% of the total proteins, the structures of only a few are known in detail. An important group of integral membrane proteins are ion-transporting ATPases, which shares the formation of an acid-stable phosphorylated intermediate as part of their reaction cycle (Jennings, 1976; Mangialavori et al., 2011).

The red blood cell (RBC) is, both structurally and metabolically, the simplest cell in the body and the end product of the maturation of bone-marrow reticulocytes. During its maturation, the RBC loses all its subcellular organelles. RBC represents 40-45% of blood volume and over 90% of the formed elements (erythrocytes, leukocytes, and platelets) in blood (Seifter et al., 2005). The biconcave

shape of the red blood cell provides an optimal area for respiratory exchange (Wielders, 2012). The latter requires passage through microcapillaries, which is achieved by a drastic modification of its biconcave shape, made possible only by the loss of the nucleus and cytoplasmic organelles and, consequently, the ability to synthesize proteins (Hajjawi, 2012b).

Without nuclei, it lacks the ability to synthesize deoxyribonucleic acid (DNA) or ribonucleic acid (RNA). Without ribosomes or an endoplasmic reticulum, it cannot synthesize or secrete protein. Because it cannot oxidize fats, a process requiring mitochondrial activity, the RBC relies exclusively on blood glucose as a fuel. Metabolism of glucose in the RBC is entirely anaerobic, consistent with the primary role of the RBC in oxygen transport and delivery, rather than its utilization (Nelson and Cox, 2008). In the red cell, 10-20% of the glycolytic intermediate, 1,3-bisphosphoglycerate, is diverted to the synthesis of 2,3-bisphosphoglycerate (2,3-BPG), an allosteric regulator of the O_2 affinity of haemoglobin (Hb) (van Wijk, and van Solinge ,2005). The pentose phosphate pathway, a shunt from glycolysis, accounts for about 10% of glucose metabolism in the red cell. In the red cell this pathway has a special role in protection against oxidative stress, while in nucleated it also serves as a source of nicotinamide adenine dinucleotide phosphate (NADPH) a reducing agent for biosynthetic reactions and pentoses for nucleic acid synthesis (Berthon et al., 1992; Nakayama et al., 2005; Hajjawi, 2012b).

Mediated Permeation

The human RBC has a high intracellular concentration of K^+ (140 mM) against extracellular concentration of 4.0 mM , and low intracellular concentration of Na^+ (140 mM) against extracellular concentration of 16 mM (Table 1). These ionic gradients are generated by a specific transport system, Na^+/K^+- ATPase that was discovered in the human RBC by Post et a. (1960). The accuracy of measuring influx and efflux of Na^+ and K^+ has been greatly enhanced by using the radioactive isotopes of $^{24}Na^+$ and $^{42}K^+$ (Glynn, 1956). Also, studies with ^{32}P-labelled ATP of plasma membrane have demonstrated that cations transport and ATPase activity were expressed when both Na^+ and K^+ are present (Jennings, 1976). This Na^+ / K^+ -dependent ATPase is inhibited by bumetanide (Lubowitz, 1977), furosemide (Russell, 2000) , dipyridamole (Joiner et al., 2001) and by ouabain (Gardner and Conlon, 1972; Dunn, 1973; Ellory et al., 1983). Such activities closely parallel the Na / K pumping activity of RBC. Its special requirements are identical to those of the pump: it interacts with Mg^{2+} , ATP, Na^+ and Ca^{2+} at the cytoplasmic surface of the membrane and with K^+ and the inhibitor at the outer surface (Saris et al., 2000). Experimental reversal of the normal ion gradients across a sealed red blood cell membrane, i.e. to high Na inside and high K outside (Table 1), provides an energy supply with witch this activity can be driven backwards to synthesize ATP within the ghost , a degenerating or fragmented RBC with no hemoglobin. It has therefore been concluded that this ATPase activity represents the enzyme expression of the energy supply to the transport of Na^+ and K^+ (Hoffman, 2008).

Table 1: Distribution of ions across the human red cell membrane.
The RBC membrane separates two compartments of cations and anions different concentrations, resulting in differences in ion potential across the membrane The ions are exchanged according to uphill concentration gradient mechanism (Eq.6).
Adapted from: Hladky,S.B. and Pink, T.J. (1977) pH Equilibrium Across the Red Cell Membrane, in Ellory, J.C. and Lew,V.L. (eds.), Membrane Transport in Red Cells, p.120.London: Academic Press Inc. (London) Ltd.

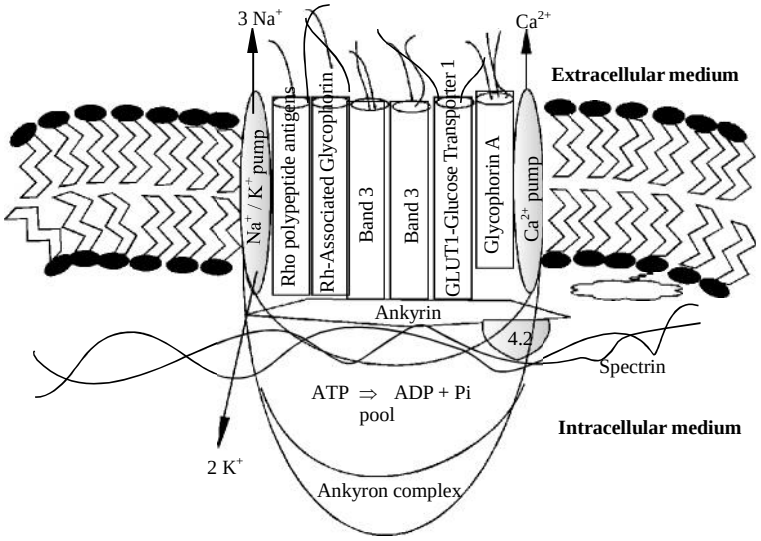
Ion	[Intracellular] mM	[Extracellular] mM	ln $[C]_{high}/[C]_{low}$	Calculated Ψ(mV)
Na^+	16	140	2.169	51.1
K^+	140	4	3.555	7790
Mg^{2+}	0.5	2	1.386	16.3
Ca^{2+}	10^{-4}	2	9.903	116.5
H^+	$7x10^{-5}$	$4x10^{-5}$	$5.600x10^{-1}$	13.2

Table 1: Distribution of ions across the human red cell membrane. - continued

HCO_3^-	16	24.8	$4.380x10^{-1}$	10.3
Cl^-	77	115	$4.010\ x10^{-1}$	9.4
OH^-	$1.6x10^{-7}$	$.5x10^{-7}$	$4.446\ x10^{-1}$	10.5
pH	7.2	7.4		

Internal Na^+ stimulates phophorylation and external K^+ stimulates an inhibitor- sensitive

Figure 2: Counter-transport of Na^+ and K^+ in human red blood cells.
ATPase pump is present in 4 conformational states, namely: $E_{1intracellular}$, $E_{1intracellular} - P_i$, $E_{2extracellular}$, $E_{2extracellular} - P_i$. $E_{1intracellular}$ loads $3Na^+_{in}$, $E_{1intracellular} - P_i$ catalyzes ATP ↔ ADP exchange reaction, $E_{2extracellular} - P_i$ allows access of $2K^+_{out}$ by catalyzing P_i ↔ H-OH exchange reaction, $E_{2extracellular}$ loads $2K^+_{out}$. The transport cycle involves the transient formation of two occluded states: $E_{1intracellular}$ - $3Na^+_{in}$ and $E_{2extracellular}$ $2K^+_{out}$.
Adapted from: Chu,H.,Puchulu-Campanella,E.,Galan,J.A.,Tao,W.A., Low,P.S. and Hoffman, J.F. (2012)"Identification of cytoskeletal elements enclosing the ATP pools that fuel human red blood cell membrane cation pumps",PNAS,vol.10, pp.1073-1083.



dephosphorylation. $3Na^+$ is effluxed concomitantly with phosphorylation and $2K^+$ influx occurs simultaneously with dephosphorylation through membrane transporter (Fig.2).

Definition of the asymmetric nature of the action of the Na^+ / K^+ pump was achieved in resealed RBC ghosts (Duhl and Hokin1974; Kaestner and Bernhardt 2002). The key functions of the Na^+ , K^+ , ATPase include: (1) to establish an intracellular ion environment high in $[K^+]$ and low in $[Na^+]$, (2) to control cell volume as revealed by the swelling of RBCs on inhibition of Na^+ / K^+ pump, (3) to provide electrochemical Na^+ gradient for driving secondary active transport system, (4) to establish a resting membrane potential (a) directly via effluxing excess positive charge , and (b) indirectly via the ion concentration gradients it creates (Hoffman et al., 2003; Rinehart et al., 2010).

In the RBC ghosts three Na^+ were transported outwards and two K^+ inwards as a result of the hydrolysis of one ATP molecule at the inner surface of the membrane. Pumping is inhibited by ouabain, furosemide, bumetanide and others (Ellory et al., 1983; Russell, 2000; Graves, 2007) at the outer surface of the membrane and by Ca^{2+} at the cytoplasmic surface (lindner et al., 1992). The hydrolysis of ATP by this system suggested that components of the active transport system might be recognized by their possession of ATPase activity. Internal Na^+ stimulates phosphorylation and external K^+ stimulates an "ouabain"-sensitive dephosphorylation. The enzyme shows K^+ stimulated phosphatase activity against extracellular *p*-nitrophenylphosphate (Garraham and Rega, 1972; Hermle et al., 2006). Furthermore, the Cl^- permeability is about 2 million fold higher than corresponding permeability of Na^+ (3.8 m.eq/ litre cells hr) with an appearant activation energy ~ 83 kJ/mol and of

K^+ (1.67 m.eq/ litre cells hr) with an appearant activation energy of ~51kJ/mol (Solomon, 1952; Knauf, 1979).

The Na^+/K^+–ATPase protein has been isolated and the usual techniques have involved removal of extrinsic proteins with chaotropic agents (Salvi and Rios, 2005) followed by selective extraction of intrinsic proteins with mild detergents (Gunn, 1973; Knauf et al., 1974; Gatto and Milanick, 2009). All pure preparations have similar specific activities, and they always possess two types of polypeptide components. The larger α polypeptide has a molecular weight of about 112.5 kDa; it contains a relatively large proportion of non-polar amino-acid residues and is reversibly phosphorylated and dephoshporylated at the β-carboxyl of a single aspartyl residue (Köksoy, 2002). It also bears a binding site for ouabain and this, taken together with its phosphorylation, indicates that *in situ* this polypeptide must be in contact with both intracellular and extracellular compartment. The smaller polypeptide (~ 45kDa) is glycosylate that seems to be an essential component of the ATPase since antibodies against it inhibit ATPase activity, and cross-linking indicates that it is in close proximity with the catalytic subunit (Grüber, 2001).

Several of the proteins involved in mediated permeation have already been positively identified as spanning proteins, some of them with substantial glycopeptides segments which could stabilize them in a single orientation in the membrane: this is the likely function of the glycopolypeptide of the Na^+ / K^+ dependent ATPase (Jørgensen, 1982). The complete amino acid structure for the α subunit of human Na / K –ATPase has been determined (Kawakami et al., 1986); its structure is compatible with a protein structure that includes 8 membrane- spanning domains to make the α subunit an integral membrane protein (Shull and Lingrel, 1987). Such spanning molecules almost certainly provide a controlled pathway for permeants through the lipid barrier. The primary structure of the β subunit has also been characterized, and it is an integral membrane protein with at least one transmembrane domain (Kawakami et a.,1986; Mercer et al., 1986). Most of the β subunit protein appears to be extracellular, whereas the non membrane-spanning domains of the α subunit are primarily intracellular. The Na^+/K^+–ATPase may exist as oligomers of the α, β and γ subunits in a ratio of 1:1:1.

The provision of energy supply to active transport systems can take several forms. Many systems use an electrochemical gradient of protons (proton motive force), generated through redox or ATP0hydrolysing reactions, to drive protons – (or hydroxyl -) linked symport or antiport systems, whilst others derive the energy for the reaction of ion gradients (e.g. of Na^+ or Ca^{2+}) directly from the hydrolysis of ATP (Boyer, 1993; Wijk and van Solinge, 2005). Ion gradients that are established in these ways then form a reservoir of energy that can be used by additional active transport systems which promote antiport or symport of permeants coupled to the ions of preformed gradients. In some systems the membrane potential created by ion asymmetries is used directly to drive particular ions through specific transport systems against their own concentration gradients, thus tending to collapse the membrane potential (Hladky and Rink, 1976; Gedde and Huestis, 1997; Nwafor and Coakley, 2003).

The provision of energy for synthesis is probably the most important function of the $\Delta\bar{\mu}_{H^+}$ across their energy coupling membranes, and maintenance of their normal [ATP]/ [ADP] ratios:

$$ADP^{3-} + H_2PO_4^- \leftrightarrow ATP^{4-} + H_2O \qquad (8)$$

Synthesis of 1 mol of ATP by these membranes appears to require the translocation of 2 mol of H^+ down $\Delta\bar{\mu}_{H^+}$. This reaction may be written:

$$ADP^{3-} + H_2PO_4^- + 2H_I^+ \leftrightarrow ATP^{4-} + H_2O + 2H_{II}^+ \qquad (9)$$

where H_I^+ and H_{II}^+ represent before and after they have crossed the membrane compartments down their electrochemical gradient, respectively (Kennedy et al., 1986; Chu et al., 2012).

This synthesis is catalyzed in the forward direction by ATP synthase, whilst in the reverse direction it is an ATPase (specifically distinguished as 'coupling ATPase', H^+-ATPase or proton-translocating (Mg^{2+}) ATPase).

ATP forms the major chemical energy currency from the energy coupling membranes to other intracellular compartments, where one of its most important uses is to drive membrane ion pumps.

These transport ATPases differ from the H^+-translocating ATPase in that their reaction mechanisms appear generally to involve a cycle of phosphorylation and dephosphorylation of the transport protein. Under normal physiological conditions in which abnormality high gradients are imposed their reactions can be reversed so as to synthesize ATP (Lohman et al., 2012).

Plasma membrane maintains intracellular Ca^{2+} ion concentration of 100 nm or lower in the face of an extracellular concentration of about 1.5 mM (Folk and Strunecka, 1990). The maintenance of this gradient is probably largely the responsibility of the Ca^{2+}- pump ATPase found in the plasma membrane. This activity can be distinguished from other ATPase by its inhibitor specificity; it is inhibited by thiol reagents and by ethacrynic acid (Reed et al., 1990), but insensitive to ruthenium red and ouabain which inhibits Na^+ / K^+ –ATPase (Lindner et al., 1992; Bernhardt et al. 1999). Maintenance of a low intracellular Ca^{2+} concentration appears to be essential for the normal functioning of some intracellular enzymes (Carafoli, 1991). In addition, the existence of a substantial gradient of Ca^{2+} concentration allows changes in membrane Ca^{2+} permeability to be utilized by changing the electrical properties of the membranes (Table 1). The other major gradients at membrane surface is Na^+ and K^+ gradients set up in opposite directions by Na^+/ K^+-ATPase (Table 1). Pumping of these two ions appears to be stoichiometrically equivalent, i.e. electroneutral, whilst it is electrogenic, with stoichiometries of up to $3Na^+ : 2K^+$ (Fig.2). The high intracellular K^+ concentration is achieved by this pump that seems necessary for certain intracellular enzyme reactions, e.g. glyceraldehyde-3-phosphate dehydrogenase in the glygolytic pathway (Sachs, 1981). Table 1 shows the ion gradients which are essential for the occurrence of many bioelectric phenomena and to supply the energy for the active accumulation of other molecules.

The osmotic gradient created by separating haemoglobin from the plasma drives H_2O into the cell, and the electro gradient established by impermanent anions of haemoglobin creates Donnan equilibrium (Bisognano et al., 1993; Hajjawi and Hider, 2009) that also contribute to an increase in intracellular H_2O. The RBC has to compensate for intracellular induced H_2O to prevent haemolysis through the disequilibrium of the permanent cations (De Franceschi and Beuzard, 1998; Hajjawi, 2010). The rates of activities of Na^+/Li^+ are elevated in red blood cells with a consistent concomitant from patients with essential hypertension as well as with insulin-dependent diabetes mellitus (Brent et al., 1989; Canessa et al., 1992). Nephropathy has suggested the hypothesis that a predisposition to essential hypertension may be the factor that, along with hyperglycemia, underlies the development of diabetic nephropathy (Zerbini et al., 2004).

Conclusion

The elegant membrane system in human RBCs depends on the energy contained in ATP which is generated during cellular oxidation of glucose to carbon dioxide and water (Eq.3). Hence, although human RBC sugar transport displays a kinetic complexity that is not explained by available models for carrier-mediated facilitated diffusion (Cloherty et al., 1996) , glucose is the main cellular metabolic fuel and glucose homeostasis is essential (Lew et al., 2007). Blood glucose concentration is normally maintained within a narrow range of 4.0-6.0 mmol/l (72-108 mg/dl) despite the fluctuation in glucose intake and its cellular utilization (Khera et al., 2008), and both reduced blood glucose (hypoglycaemia) and increased blood glucose (hyperglycaemia) threaten health. The absorption of glucose occurs via a cotransport (or symport) mechanism linked to the absorption of Na^+. This process is considered secondary active transport, because the absorption of the sugars is dependent on the sodium gradient established by the Na^+ / K^+ pump, rather than ATP directly; the process is considered active because ATP is required to fuel the pump that generates the Na^+ gradient (Motais and Sola, 1973; Leitch and Carruthers, 2007).

The disequilibrium of cations and anions in RBC conserve discocyte shape of human RBCs (~7.5 to 8.7 μm in diameter and 1.7 to 2.2 μm in thickness) (Nelson and Cox, 2008), in which the ATPase activity in intact RBCs functions as an energy reserve to sustain ion pumping attributes. Also, the functional RBC passage through microcapillaries and spleen sinusoids is a complicated

dynamics process involving repeated deformations. Hence, disruption of RBC morphology and mechanics can be critically affected by genetic and acquired pathological conditions (Bernhardt and Ellory, 2003).

References

[1] Bernhardt, I., Bogdanova, A. Y., Kummerowi, D., Kiessling, K., Harmann, J.and Ellory, J. C. (1999) "Characterization of the K+ (Na+)/H+ monovalent cation exchanger in the human red blood cell membrane: effects of transport inhibitors", Gen. Physiol. Biophys. , vol.18, pp.119—137.

[2] Bernhardt, I. and Ellory, J. C. (2003) Red Cell Membrane Transport in Health and Disease. Berlin: Springer-Verlag.

[3] Berthon, H.A., Kuchel, P.W. and Nixon, P.F. (1992) "High control coefficient of transketolase in the nonoxidative pentose phosphate pathway of human erythrocytes: NMR, antibody, and computer simulation studies", Biochemistry, vol.31 (51), pp.12792-12798.

[4] Bisognano, J.D., Dix, J.A., Pratap, P.R., Novax, T.S. and Freedman, J.C. (1993) "Proton (or hydroxide) fluxes and the biphasic osmotic response of human red blood cells", J.Gen.Physiol, vol.102, pp.99-123.

[5] Boyer, P.D. (1993) "The binding change mechanism for ATP synthase- some probabilities and possibilities", Biochim. Biophys. Acta, vol.1140, pp.215-250.

[6] Brent, G.A., Canessa, M. and Dluhy, R.G (1989) "Reversible alteration of red-cell lithium-sodium countertransport in patients with thyroid disease", J. Clin, Endocrinol. Metab., vol. 68, pp. 322–328.

[7] Brugnara, C. (2003) "Sickle cell disease: from membrane pathophysiology to novel therapies for prevention of erythrocyte dehydration", J.Pediatr. Hematol., vol.25, pp.927-933.

[8] Diez-Silva, M., Dao, M., Han, J., Lim,C.T., and Suresh, S. (2010) " Shape and biomechanical characteristics of human red blood cells in health and disease", MRS Bull., vol. 35(5), pp. 382–388.

[9] Canessa, M., Zerbini, G. and Laffel, L.M. (1992) "Sodium activation kinetics of red blood cell Na^+/Li^+ countertransport in diabetes: methodology and controversy", J. Am. Soc. Nephrol, vol.3 (4), pp.41-49.

[10] Carafoli, E. (1991) "Calcium pump of the plasma membrane", Physiological Reviews, vol.71 (1), pp.129-153.

[11] Chu, H., Campanella, E.P., Galan, J.A., Tao, W.A., Low, P.S. and Hoffman, J.F. (2012)"Identification of cytoskeletal elements enclosing the ATP pools that fuel human red blood cell membrane cation pumps", PNAS, vol.10, pp.1073-1083.

[12] Cloherty, E.K.**,** Heard, K.S. and Carruthers, A. (1996) "Human erythrocyte sugar transport is incompatible with available carrier models", Biochemistry, vol. 35, pp.10411–10421.

[13] De Franceschi, L. and Beuzard, Y. (1998) "Red blood cell indices, cation content, and membrane cation transports", Hemoglobin, vol.22, pp.493–500.

[14] Dudzinska, W., Lubkowska, A., Dolegowska, B. , Safranow, K. and Jakubowska, K. (2010) " Adenine, guanine and pyridine nucleotides in blood during physical exercise and restitution in healthy subjects", Euro. J. Appl. Physiol., vol.110 (6), pp.1155–1162.

[15] Duhl, J.L. and Hokin, L.E. (1974) "The sodium-potassium adenosinetriphosphatase", Annual Review of Biochemistry, vol. 43, pp. 327-356.

[16] Dunn, M.J. (1973) "Ouabain-uninhibited sodium transport in human erythrocytes. Evidence against a second pump", J. Clin. Invest., vol.52 (3), pp. 658–670.

[17] Ellory, J.C., Flatman, P.W. and Stewart, G.W. (1983) "Inhibition of human red cell sodium and potassium transport by divalent cations", J.Physiol., vol.340, pp.1-17.

[18] Fiske, C.H. and Subbarow, Y. (1929) "Phosphorous compounds of muscle and liver", Science, vol. 70, pp.381–382.

[19] Folk, P. and Strunecka, A. (1990) "Calcium affects phosphoinositide turnover in human erythrocytes", Gen. Physiol. Biophys., vol. 9, pp. 281-290.

[20] Gardner, J.D. and Conlonn, T.P. (1972) "The effects of sodium and potassium on ouabain binding by human erythrocytes", J.General Physiol., vol.60, pp.609-629.

[21] Garraham, P.J. and Rega, A.F. (1972) "Potassium activated phosphatase from human red blood cells. The effects of p-nitrophenylphosphate on carbon fluxes", J.Physiol., vol.223 (2), pp.595-617.

[22] Gatto, C. and Milanick, M. (2009) "Red blood cell Na pump: insights from species differences", Blood Cells Mol.Dis., vol.42 (3), pp.192-200.

[23] Gedde, M.M. and Huestis, W.H. (1997) "Membrane potential and human erythrocyte shape", Biophys. J., vol.72 (3), pp. 1220–1233.

[24] Gibson, J.S. and Ellory, J.C. (2003) K – Cl Cotransport in Vertebrate Red Cells in Red Cell Membrane Transport in Health and Disease, Bernhardt, I. and Ellory, J.C. (eds.), pp. 197-220. Berlin: Springer-Verlog.

[25] Gibson, J.S., Stewart, G.W. and Ellory, J.C.(2000)"Effect of dimethyl adipimidate on K^+ transport and shape change in red blood cells from sickle cell patients", FEBS Lett, vol.480, pp.179-183.

[26] Glynn, L.M. (156) "Sodium potassium movements in human red cells", J. Physiol, vol.134. pp.278-310.

[27] Graves, S.W. (2007 "Sodium regulation, sodium pump function and sodium pump inhibitors in uncomplicated pregnancy and preeclampsia", Front Biosci. , vol.12, pp.2438–2446.

[28] Grüber, G., Wieczorek, H., Harvey, W.R. and Müller, V.(2001) "Structure-function relationships of A-, F- and V-ATPases", J. Exp. Biol. , vol.204(15), pp.2597-605.

[29] Gunn, R.B. (1972) "A titratable carrier model for both mono- and divalent anion transport in human red blood cells", in Oxygen Affinity of Hemoglobin and Red Cell Acid-Base Status, Rorth, M. and Astrup P. (eds.) , pp.823-827. Copenhagen: Munksgaard.

[30] Gusev, G.P. and Ivanova, T.I. (2004) " Amiloride-sensitive sodium transport in lamprey red blood cells: evidence for two distinct transport pathways", Gen. Physiol. Biophys. , vol.23, pp.443-456.

[31] Hajjawi, O.S. and Hider, R. C. (2009) "Asymmetry of the malonate transport system in human red blood cells", European Journal of Scientific Research, vol.31 (4), pp.534-545.

[32] Hajjawi, O.S. (2010) "Sequencial mechanism of the malonate transport system in human red blood cells", European Journal of Scientific Research, vol.46 (1), pp. 18-27.

[33] Hajjawi, O.S. (2011) "Succinate dehydrogenase: assembly, regulation and role in human disease", European Journal of Scientific Research, vol.51 (1), pp. 133-142.

[34] Hajjawi, O.S. (2012a) "Acetylcholinesterase in human red blood cells", European Journal of Scientific Research, vol.75 (4), pp. 510-522.

[35] Hajjawi, O.S. (2012b) Introduction to Human Red Blood Cells, in The Blood Cells, Kaestner, L. (ed.), in print. Cheyenne, WY : Academy Publishing Services LCC.

[36] Hardwicke, P.M.D. and Freytag, J.W. (1981) "A proteolipid associated with Na^+ / K^+ – ATPase is not essential for ATPase activity", Biochem. Biophys. Res. Commum., vol.102, pp.250-257.

[37] Henkelman, S., Dijkstra-Tiekstra, M.J., de Wildt-Eggen, J., Graaff, R., Rakhorst, G., and van Oeveren, W. (2010) "Is red blood cell rheology preserved during routine blood bank storage?", Transfusion, vol. 50 (4), pp.941-948.

[38] Hemle,T., Shumilina, E., Attanasio, P., Akel,, A., Kempe, D.S., Lang, P.A., Podolski,,M., Gatz, S., Bachmann, R., Bachmann,, C., Abele, H., Huber, S., Wieder,T. and Lang, F. (2006) " Decreased cation channel activity and blunted channel-dependent eryptosis in neonatal erythrocytes", Am. J. Physiol. Cell Physiol., vol. 291no. 4 , pp.C710-C717.

[39] Hladky, S. B. and Rink, T. J. (1976) "Potential difference and the distribution of ions across the human red blood cell membrane; a study of the mechanism by which the fluorescent cation, diS-C3-(5) reports membrane potential", J. Physiol., vol. 263, pp. 287-319.

[40] Hladky, S.B. and Pink, T.J. (1977) pH Equilibrium Across the Red Cell Membrane, in Ellory, J.C. and Lew, V.L. (eds.), Membrane Transport in Red Cells, p.120. London: Academic Press Inc. (London) Ltd.

[41] Hoffman, J.F. (2008)" My passion and passages with red blood cells", Annual Review of Physiology, vol. 70, pp. 1-22.

[42] Hoffman, J.F., Joiner, W., Nehrke, K., Potapova, O., Foye, K.and Wickrema, A.(2003) " The hSK4 (KCNN4) isoform is the Ca^{2+}-activated K^+ channel (Gardos channel) in human red blood cells", Proc. Natl. Acad. Sci. U. S. A., vol.100, pp. 7366– 7371.

[43] Huber, S.M., Gamper, N., and Lang, F. (2001) "Chloride conductance and volume- regulatory nonselective cation conductance in human red blood cell ghosts", Pflugers Arch. , vol.441(4), pp.551-8.

[44] Jennings, M.L. (1976) "Proton fluxes associated with erythrocyte membrane anion exchange", J. Membr. Biol., vol.28, pp.187–205.

[45] Joiner, C.H., Jiang,M., Claussen,W.J., Roszell,N.J., Yasin,Z. and Franco, R.S. (2001) "Dipyridamole inhibits sickling-induced cation fluxes in sickle red blood cells", Blood, vol.97 (12), pp.3976-3983.

[46] Jørgensen, P.L. (1982) "Mechanism of the Na+, K+ pump. Protein structure and conformations of the pure (Na+ +K+)-ATPase", Biochim. Biophys. Acta. , vol.694 (1), pp.27–68.

[47] Kaestner, L. and Bernhardt, I. (2002) "Ion channels in the human red blood cell membrane: their further investigation and physiological relevance", Bioelectrochemistry, vol.55 (1-2), pp. 71-74.

[48] Kaššák, P., Šikurovă, L., Kvasnička, P. and Bryszewska, M. (2006)"The response of Na^+/K^+-ATPase of human erythrocytes to green laser light treatment, Physiol. Research., vol.55, pp.189-194.

[49] Kawakami, K., Ohta, T., Nojima, H. and Nagano, K. (1986) "Primary structure of the alpha-subunit of human Na, K-ATPase deduced from cDNA sequence", J. Biochem. , vol.100 (2), pp.389–397.

[50] Kennedy, B.G., Lunn, G. and Hoffman, J.F. (1986) "Effects of attaining the ATP/ADP ratio on pump-mediated Na/K and Na/Na exchanges in the resealed human red blood cell ghosts", J.Gen.Physiol., vol.87 (1), pp.47-72.

[51] Khera,P.K.,Joiner,C.H., Carruthers, A., Lindsell, C.J., Smith, E.P., Franco, R.S., Holmes, Y.R. and Cohen, R.M. (2008) "Evidence for interindividual heterogeneity in the glucose gradient across the human red blood cell membrane and its relationship to hemoglobin glycation", Diabetes, vol.57 (9), pp.2445-2452.

[52] Knauf, P.A. (1979) "Erythrocyte anion exchange and the band 3 protein", Current Topics in Membranes and Transport, vol.12, pp.249-363.

[53] Knauf, P.A., Proverbio, F., and Hoffman, J.F. (1974) "Electrophoretic separation of different phophosproteins associated with Ca-ATPase and Na, K-ATPase in human red cell ghosts", J.General Physiol., vol.63, pp.324-336.

[54] Köksoy, A.Y. (2002) "Na^+ / K^+ – ATPase: a review", J.Ankara Med. Sc.,vol. 24 (2), pp.73-82.

[55] Laidler, K.J. (1987) Chemical Kinetics, 3rd edn. New York: Harper Collins.

[56] Leitch, J.M. and Carruthers, A. (2007) "ATP-dependent sugar transport complexity in human erythrocytes", Am.J.Physiol. Cell Physiol., vol.292 (2), pp.974-986.

[57] Lew, V.L., Daw, N., Perdomo, D., Etzion, Z., Bookchin, R.M. and Tiffert, T. (2003)" Distribution of plasma membrane Ca^{2+} pump activity in normal human red blood cells", Blood, vol. 102(12), pp.4206-4213.

[58] Lew, V.L., Daw, N., Etzion, Z., Tiffert, T., Muoma, A., Vanagas, L. and Bookchin, R.M. (2007) "Effects of age-dependent membrane transport changes on the homeostasis of senescent human red blood cells", Blood, vo. 110(4), pp.1334-1342.

[59] Lindner, A., Gagne, E.R., Zingraff, J., Jungers, P., Drüeke, T.B., Hannaert, P. and Garay, R. (1992) "A circulating inhibitor of the RBC membrane calcium pump in chronic renal failure", Kidney International, vol. 42, pp.1328–1335.

[60] Lipman, F. (1941) "Metabolic generation and utilization of phosphate bond energy. Adv. Enzymol., vol. 1, pp.99–162.

[61] Lohman, A.W., Billaud, M. and Isakson, B.E. (2012) "Mechanism of ATP release and signaling in the blood vessel wall", Cardiovasc. Res., vol.95 (3), pp.269-280.

[62] Lohmann, K. (1929) "Uber die pyrophosphatfraktion im muskel", Naturwissenschaften, vol. 17, pp.624–625.

[63] Lubowitz, H. (1977) "The effect of bumetanide on cation transport in human red blood cells", J. Pharmacol. Exp. Ther., vol. 203 (1), pp.92-96.

[64] Mangialavori, I.C, Caride, A. J., Rossi, R. C., Rossi, J. P.F.C. and Strehler, E. E. (2011) "Diving into the lipid bilayer to investigate the transmembrane organization and conformational state transitions of P-type ion ATPases", Current Chemical Biology, vol. 5 (2), pp. 118-129

[65] Maruyama, K. (1991) "The discovery of adenosine triphosphate and the establishment of it's structure, J. Hist. Biol., vol. 24, pp.145-154.

[66] Mercer, R.W. and Dunham, P.B. (1981) "Membrane-bound ATP fuels the Na/K pump", J.Gen.Physiol., vol.78, pp.547-568.

[67] Mercer, R.W., Schneider, J.W., Savitz, A., Emanuel, J., Benz, E.J., Jr. and Levenson, R. (1986)"Rat-brain Na,K-ATPase beta-chain gene: primary structure, tissue- specific expression and amplification in ouabain-resistant HeLa C+ cells", Mol. Cell Biol., vol.6(11), pp.3884–3890.

[68] Mishra, P.K. and Passow, R.K. (1969) "Induction of intracellular ATP synthesis by extracellular ferricyanide in human red blood cells", J.Mem.Biol., vol.1, pp.214-224.

[69] Motais, R. and Sola, F. (1973) "Characteristics of a sulphydryl group essential for sodium exchange diffusion in beef erythrocytes", J.Physiol., vol.233 (2), pp.423-438.

[70] Nakayama, Y., Kinoshita, A. and Tomita, M. (2005) "Dynamic simulation of red blood cell metabolism and its application to the analysis of a pathological condition", Theoretical Biology and Medical Modelling, vol.2, pp.18-29.

[71] Nelson, D.L. and Cox, M.M. (2008) Lehninger Principles of Biochemistry, 5th edn. New York: W.H.Freeman and Company.

[72] Nwafor, A. and Coakley, W.T. (2003) "Membrane potential change effects on cationic and neutral drug-induced erythrocyte shape change and cellular uptake of drugs", African J. Biol. Res., vol. 6 (1), pp. 9-14.

[73] Oliveira, G.A., Sousa, C.R., Da Poian, A.T., and Luz, M.R.M.P. (2003) "Students' misconception about energy-yielding metabolism: glucose as the sole metabolism fuel", Adv. Physiol. Edu., vol.27 (3), pp.97-101.

[74] Park, Y., Best, C.A., Auth, T., Gov, N.S., Safran, S.A., Popescu, G., Suresh, S. and Feld, M.S. (2010) "Metabolic remodeling of the human red blood cell membrane", PNAS, vol.107 (4), pp.1289-1294.

[75] Passow, H. (1986) "Molecular aspects of band 3 protein-mediated anion transport across the red blood cell membrane", Rev.Physiol.Biochem. Pharmacol., vol.103, pp.61-203.

[76] Perrot, P. (1998) A to Z of Thermodynamics. New York: Oxford University Press.

[77] Pilkis, S. J. and Claus, T.H. (1991) "Hepatic gluconeogenesis/glycolysis: regulations and structure/function relationships of substrate cycle enzymes", Ann. Rev.Nutrit., vol.11, pp.465-515.

[78] Post, R.L., Merrit, C.R., Kinsolving, C.R. and Albright, C.D. (1960) "Membrane adenosine triphosphatase as a participant in the active transport of sodium and potassium in the human erythrocyte", J.Biol. Che., vol.235, pp.1796-1802.

[79] Reed. D.J., Pascoe, G. A., Thomas, C.E. (1990) "Extracellular calcium effects on cellular viability and thiol homeostasis", Environmental Health Perspectives, vo.81, pp. 113-120.

[80] Rinehart, J., Gulcicek, E.E., Joiner, C.H., Lifton, R.P. and Gallagher, P.G. (2010) "Determinants of erythrocyte hydration", Curr. Opin. Hematol., vol.17 (3), pp.191-7.

[81] Romero, P.J. and Romero, E.A. (2003)"New vanadate-induced Ca pathway in human red cells", Cell Biol. Int., vol.27, pp.903-912.

[82] Russell, J.M. (2000) "Sodium-Potassium-Chloride Cotransport", Physiol Review, vol.80 (1), pp.211-276.

[83] Sachs, J.R.(2003) Na^+ / K^+ Pump in Red Cell Membrane Transport in Health and Disease, Bernhardt, I. and Ellory, J.C. (eds.), pp. 111-137. Berlin: Springer-Verlog.

[84] Salvi, G. and Rios, P. L. (2005) "Effective interactions between chaotropic agents and proteins", Proteins: Structure, Function and Bioinformatics, vol.61 (3), pp.492-499.

[85] Saris, N.E.L., Mervaala, E., Karppanena, H., Khawaja, J.A., and Lewenstam, A. (2000) "Magnesium an update on physiological, clinical and analytical aspects",Clinica Chimica Acta , vol.294, pp.1–26.

[86] Scott, C. (2005) "Misconception about aerobic and anaerobic energy expenditure ", J. Int. Sports Nutrition, vol. 3, pp.32-37.

[87] Seifter, J., Ratner, A. and Sloane, D. (2005) Concepts in Medical Physiology. Baltimore, MD: Lippincott Williams & Wilkins.

[88] Shull, M.M. and Lingrel, J.B. (1987) "Multiple genes encode the human Na+,K+- ATPase catalytic subunit", Proc. Natl. Acad. Sci. U. S. A., vol.84 (12), pp.4039–4043.

[89] Solomon, A. K. (1952) "The permeability of the human erythrocyte to sodium and potassium", J. Gen. Physiol, vol.36 (1), pp.57-100.

[90] Van Wijk, R. and van Solinge, W.W. (2005) "The energy-less red blood cell is lost: erythrocyte enzyme abnormalities of glycolysis", Blood, vol.106 (13), pp.4034-4042.

[91] Wielders, J.P.M. (2012) "Vitamin D, myth or miracle?", Ned Tijdschr Klin Chem Labgeneesk , vol. 37 (1), pp. 76-79.

[92] Zerbini, G, Gabellini, D., Ruggieri, D. and Maestroni, A. (2004) "Increased sodium- lithium countertransport: a cellular dysfunction common to essential hypertension and diabetic nephropathy", J. Am. Soc. Nephrology, vol.15 (1), pp.81-84.