The energy-less red blood cell is lost – erythrocyte enzyme abnormalities of glycolysis

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Abstract

The red blood cell depends solely on the anaerobic conversion of glucose by the Embden-Meyerhof Pathway for the generation and storage of high-energy phosphates, which is necessary for the maintenance of a number of vital functions. Many red blood cell enzymopathies have been described that disturb the erythrocyte’s integrity, shorten its cellular survival, and result in hemolytic anemia. By far the majority of these enzymopathies are hereditary in nature. In this review, we summarize the current knowledge regarding the genetic, biochemical, and structural features of clinically relevant red blood cell enzymopathies involved in the Embden-Meyerhof Pathway and the Rapoport-Luebering Shunt.
Abbreviations

2,3-DPG, 2,3-bisphosphoglycerate
ADP, adenosine diphosphate
ATP, adenosine triphosphate
BPGM, bisphosphoglycerate mutase
CNSHA, chronic nonspherocytic hemolytic anemia
DHAP, dihydroxyacetone phosphate
F6P, fructose-6-phosphate
FBP, fructose-1,6-bisphosphate
G6P, glucose-6-phosphate
GPI, glucose 6-phosphate isomerase
HK, hexokinase
LDH, lactate dehydrogenase
MPGM, monophosphoglycerate mutase
NADH, nicotinamide adenine dinucleotide
PFK, phosphofructokinase
PGK, phosphoglycerate kinase
PK, pyruvate kinase
TPI, triosephosphate isomerase
Introduction

The moment the mature red blood cell leaves the bone marrow, it is optimally adapted to perform the binding and transport of oxygen, and its delivery to all tissues. This is the most important task of the erythrocyte during its estimated 120-day journey in the blood stream. The membrane, hemoglobin, and proteins involved in metabolic pathways of the red blood cell interact to modulate oxygen transport, protect hemoglobin from oxidant-induced damage, and maintain the osmotic environment of the cell. The biconcave shape of the red blood cell provides an optimal area for respiratory exchange. 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.\textsuperscript{1}

During their intravascular lifespan, erythrocytes require energy to maintain a number of vital cell functions. These include (1) maintenance of glycolysis; (2) maintenance of the electrolyte gradient between plasma and red cell cytoplasm through the activity of adenosine triphosphate (ATP)-driven membrane pumps; (3) synthesis of glutathione and other metabolites; (4) purine and pyrimidine metabolism; (5) maintenance of hemoglobin’s iron in its functional, reduced, ferrous state; (6) protection of metabolic enzymes, hemoglobin, and membrane proteins from oxidative denaturation; and (7) preservation of membrane phospholipid asymmetry. Because of the lack of nuclei and mitochondria, mature red blood cells are incapable of generating energy via the (oxidative) Krebs cycle. Instead, erythrocytes depend on the anaerobic conversion of glucose by the Embden-Meyerhof Pathway for the generation and storage of high-energy phosphates (Figure 1). Moreover, erythrocytes possess a
unique glycolytic bypass for the production of 2,3-bisphosphoglycerate (2,3-DPG), the Rapoport-Luebering Shunt. This shunt bypasses the phosphoglycerate kinase (PGK) step and accounts for the synthesis and regulation of 2,3-DPG levels that decreases hemoglobin’s affinity for oxygen. In addition, 2,3-DPG constitutes an energy buffer.

A number of red blood cell enzymopathies have been described in the Embden-Meyerhof Pathway. The lack of characteristic changes in red blood cell morphology differentiates the glycolytic enzymopathies from erythrocyte membrane defects and most hemoglobinopathies. In general, red blood cell enzymopathies cause chronic nonspherocytic hemolytic anemia (CNSHA), albeit to a variable degree. The continuous lack of sufficient energy and other metabolic impairments results in a shortened lifespan of the mature red blood cell. The degree of hemolysis is dependent on the relative importance of the affected enzyme and the properties of the mutant enzyme with regard to functional abnormalities or instability, or both. The ability to compensate for the enzyme deficiency by overexpressing isozymes or using alternative pathways contribute to the clinical picture of patients with red blood cell enzymopathies. A physiological response to compensate for anemia caused by hemolysis is increased erythrocyte production: reticulocytosis (reticulocytes normally comprise 0.5 to 2.5% of total erythrocytes). Reticulocytes still preserve cytoplasmic organelles, including ribosomes and mitochondria, and are thus capable of protein synthesis and the production of ATP by oxidative phosphorylation. Several enzymes including hexokinase (HK), pyruvate kinase (PK), and aldolase display much higher activity in reticulocytes and are often referred to as the age-related enzymes.

A number of red blood cell enzymopathies concern enzymes expressed in other tissues as well. In general, the deficiency is more pronounced in red blood cells, when
compared to other cells, because of the long life span of the mature erythrocyte after
the loss of protein synthesis. Therefore, once an enzyme in red blood cells is degraded
or has otherwise become nonfunctional, it cannot be replaced by newly synthesized
proteins. In addition, tissue-to-tissue differences in proteases may play a role in the
extent to which various tissues manifest the deficiency state.\textsuperscript{10} It may well be that
those mutant glycolytic enzymes that affect all tissues cause prenatal mortality and
are, therefore, never seen by clinicians. The mutant enzymes that come to light are
those in whom the distribution of the deficiency is compatible with extrauterine life.
By far the majority of red cell enzymopathies are hereditary in nature, although
acquired deficiencies have also been described, mainly in malignant hematological
disorders.\textsuperscript{11}

In this review, a summary is made of the major features regarding the biochemical,
structural, and genetic basis of clinically relevant red blood cell enzymopathies
The Embden-Meyerhof Pathway

Glucose is the energy source of the red blood cell. Under normal physiological circumstances (*i.e.* no excessive oxidative stress), 90% of glucose is catabolized anaerobically to pyruvate or lactate by the Embden-Meyerhof Pathway, or glycolysis (Figure 1). Although one mole of ATP is used by HK, and an additional mole of ATP by phosphofructokinase (PFK), the net gain is two moles of ATP per mole of glucose, because a total of four moles of ATP are generated by PGK and PK. In addition, reductive potential is generated in the form of nicotinamide adenine dinucleotide (NADH) in the step catalyzed by glyceraldehyde-3-phosphate dehydrogenase. This can be used to reduce methemoglobin to hemoglobin by NADH-cytochrome b5 reductase. If this reaction takes place, the end product of the glycolysis is pyruvate. However, if NADH is not reoxidized here, it is used in reducing pyruvate to lactate by lactate dehydrogenase (LDH) in the last step of glycolysis.

The Embden-Meyerhof Pathway is subjected to a complex mechanism of inhibiting and stimulating factors. The overall velocity of red blood cell glycolysis is regulated by three rate-limiting enzymes, HK, PFK, and PK, and by the availability of NADH and ATP. Some glycolytic enzymes are allosterically stimulated (e.g. fructose-1,6-bisphosphate (FBP) for PK) or inhibited (e.g. glucose-6-phosphate (G6P) for HK) by intermediate products of the pathway.

**Hexokinase**

Hexokinase catalyzes the phosphorylation of glucose to G6P, using ATP as a phosphoryl donor (Figure 1). As the initial step of glycolysis, HK is one of the rate-limiting enzymes of this pathway. The activity of hexokinase is significantly higher in
reticulocytes compared to mature red cells, in which it is very low. In fact, of all
glycolytic enzymes HK has the lowest enzymatic activity \textit{in vitro}.\textsuperscript{12}

In mammalian tissues four isozymes of HK with different enzymatic properties exist,
HK-I to III, with a molecular mass of 100 kDa, and HK-IV (or glucokinase), with a
molecular mass of 50 kDa. HK-I to III are considered to be evolved from an ancestral
50 kDa HK by gene duplication and fusion.\textsuperscript{13} Consequently, both the C- and N-
terminal halves of HK-I to III show extensive internal sequence similarity but only in
case of HK-II is catalytic function maintained in both the C- and N-terminal halves.
HK-I and HK-III have further evolved into enzymes with catalytic (C-terminal) and
regulatory (N-terminal) halves, respectively. HK-I is the predominant isozyme in
human tissues that depend strongly on glucose utilisation for their physiological
functioning, such as brain, muscle, and erythrocytes. HK-I displays unique regulatory
properties in its sensitivity to inhibition by physiological levels of the product G6P
and relief of this inhibition by inorganic phosphate.\textsuperscript{14,15}

The determination of the structures of the human (PDB entry 1DGK) and rat HK-I
isozymes have provided substantial insight into ligand binding sites and subsequent
modes of interaction of these ligands (Figure 2).\textsuperscript{16,17} The mode of inhibition by G6P
remains subject to debate.

Erythrocytes contain a specific subtype of HK (HK-R)\textsuperscript{18} that is encoded by the HK-I
gene (\textit{HK1}), localized on chromosome 10q22 and spanning more than 100 kb. The
structure of \textit{HK1} is complex. It encompasses 25 exons, which, by tissue-specific
transcription, generate multiple transcripts by alternative use of the 5’ exons.\textsuperscript{19}
Erythroid-specific transcriptional control results in a unique red blood cell-specific
mRNA that differs from HK-I transcripts at the 5’ untranslated region (5’-UTR) and
at the first 63 nucleotides of the coding region. Consequently, HK-R lacks the porin-binding domain that mediates HK-I binding to mitochondria.

Hexokinase deficiency (OMIM 235700) is a rare autosomal, recessively inherited disease with CNSHA as the predominant clinical feature. As with most glycolytic red cell enzyme deficiencies the severity of hemolysis is variable, ranging from severe neonatal hemolysis and death to a fully compensated chronic hemolytic anemia. Splenectomy is in general beneficial. Seventeen families with hexokinase deficiency have been described to date and only three patients have been characterized at the molecular level. One compound heterozygous patient carried a missense mutation that encoded a leucine to serine substitution at residue 529. An as yet unidentified mutation on the other allele caused skipping of the sixth exon, as detected in this patient’s cDNA. One other patient was homozygous for a missense mutation that predicted the substitution of a highly conserved threonine by serine at residue 680 in the enzyme’s active site, where it interacted with phosphate moieties of adenosine diphosphate (ADP), ATP, and G6P. The last case constituted a lethal out-of-frame deletion of exons 5 to 8 of HK1. This deletion was identified in the homozygous state in a fetus who died in utero.

In mice, a mutation associated with downeast anemia causes severe hemolytic anemia with extensive tissue iron deposition and marked reticulocytosis, closely resembling human CNSHA. The primary defect is in the murine Hk1 gene and HK activity is markedly decreased in red blood cells, spleen, and kidney. As such, it represents a mouse model of generalized HK deficiency.

Glucose-6-Phosphate Isomerase
Glucose 6-phosphate isomerase (GPI) catalyzes the interconversion of G6P into fructose-6-phosphate (F6P) in the second step of the Embden-Meyerhof Pathway (Figure 1). As a result of this reversible reaction, products of the Hexose Monophosphate Shunt can be recycled to G6P. Unlike HK and other age-related enzymes, the GPI activity in reticulocytes is only slightly higher than that of mature erythrocytes. Apart from its role in glycolysis, GPI exerts cytokine properties outside the cell and is involved in several extracellular processes. Because GPI knock-out mice die in the embryological state, GPI is considered to be a crucial enzyme. Recently, the crystal structure of human GPI was resolved (PDB entry 1IAT). The enzyme is a homodimer, composed of two 63-kDa subunits of 558 amino acids each. The active site is composed of polypeptide chains from both subunits. Thus, formation of the dimer is a prerequisite for catalytic activity. The structural gene coding for GPI (GPI) is located on chromosome 19q13.1. GPI spans at least 50 kb and consists of 18 exons that are transcribed into a cDNA of 1.9 kb in length.

GPI deficiency (OMIM 172400) is an autosomal recessive disease and is second to PK deficiency in frequency, with respect to glycolytic enzymopathies. Approximately 50 families with GPI deficiency have been described worldwide. Homozygous or compound heterozygous patients have chronic hemolytic anemia of variable severity and display enzymatic activities of less than 25% of normal. Hemolytic crises may be triggered by viral or bacterial infections. Hydrops fetalis appears more common in GPI deficiency than in other enzyme deficiencies. In rare cases, GPI deficiency also affects non-erythroid tissues, causing neurological symptoms and granulocyte dysfunction. Normally, GPI is very stable but a striking feature of nearly all GPI mutants is their thermolability, whereas kinetic properties are more or less unaffected. Twenty-nine mutations have been detected in GPI, 24 of which were missense.
mutations, three were nonsense mutations, and two mutations affected splice sites.\textsuperscript{31} Mapping of these mutations to the crystal structure of human GPI has provided insight in the molecular mechanisms causing hemolytic anemia in this disorder. In accordance with the three-dimensional structure, mutations can be categorized into three distinct groups that affect (1) the overall structure, (2) the dimer interface and (3) the active site.\textsuperscript{29}

As in humans, homozygous GPI-deficient mice exhibit severe CNSHA, reticulocytosis, and hyperbilirubinemia. The hematologic features in the mouse mutants resembles that of the severe, chronic form of the human enzymopathy with increased G6P levels, and normal or decreased concentrations of glycolytic metabolites following the metabolic block. In addition, other tissues are also affected, indicating a reduced glycolytic capability of the whole organism.\textsuperscript{34}

\textbf{Phosphofructokinase}

Phosphofructokinase catalyzes the rate-limiting, ATP-mediated phosphorylation of F6P to FBP (Figure 1). PFK is a homo- or heterotetramer with a molecular mass of approximately 380 kDa, and the enzyme is allosterically regulated, among other metabolites, by 2,3-DPG.\textsuperscript{35} Three different subunits have been identified in humans: PFK-M (muscle), PFK-L (liver) and PFK-P (platelet).\textsuperscript{36} The subunits are expressed in a tissue-specific manner and, in erythrocytes, five isoenzymes of varying subunit composition (M\textsubscript{4}, M\textsubscript{3}L\textsubscript{1}, M\textsubscript{2}L\textsubscript{2}, ML\textsubscript{3}, and L\textsubscript{4}) can be identified.\textsuperscript{37} The gene encoding the M subunit (\textit{PFKM}) has been assigned to chromosome 12q13.3 and spans 30 kb. It contains 27 exons and at least three promoter regions.\textsuperscript{38} The L-subunit encoding gene (\textit{PFKL}) is located on chromosome 21q22.3, it contains 22 exons and spans more than 28 kb.\textsuperscript{39}
PFK deficiency (OMIM 171850) is a rare autosomal, recessively inherited disorder. Because red blood cells contain both M- and L-subunits, mutations affecting either gene will affect enzyme activity. Thus, mutations concerning the L subunit will render red blood cells that contain only M₄ and they are partially PFK deficient. In such cases, patients display a mild hemolytic disorder without myopathy. Likewise, a deficiency of the M subunit results in the absence of muscle PFK and, in addition, causes PFK deficiency in erythrocytes. Accordingly, deficiency of the M subunit causes myopathy and a mild hemolytic disorder. Erythrocytes that express only the L₄ PFK also show a metabolic block at the PFK step in glycolysis and lowered 2,3-DPG levels. To date, 15 PFK deficient PFKM alleles from more than 30 families have been characterized.⁴⁰ One-third of the reported patients are of Jewish origin and in this population an intronic splice site mutation, IVS5+1G>A,⁴¹ and a single base-pair deletion in exon 22, c.2003delC,⁴² are among the most frequently encountered mutations.

A naturally occurring animal model of PFK-M deficiency has been described in English springer spaniels. These extensively studied dogs have a chronic compensated hemolytic disorder and exertional myopathy, as is seen in human patients. Molecular analysis identified a nonsense mutation in the penultimate exon of the PFK-M gene, causing the rapid degradation of the truncated PFK-M subunit.⁴³

**Aldolase**

Aldolase catalyzes the reversible conversion of FBP to glyceraldehyde-3-phosphate and dihydroxyacetone phosphate (DHAP) (Figure 1). Aldolase is a tetramer of identical subunits of 40 kDa each, and three distinct isoenzymes have been identified, aldolase A, B, and C. The 364 amino acids long aldolase A subunits are
predominantly expressed in erythrocytes and muscle. The X-ray crystallographic structure of human muscle aldolase A (PDB entries 1ALD, 2ALD, and 4ALD) demonstrates that the homotetrameric enzyme is composed of four monomers, each characterized by an eight-membered αβ-barrel structure. The active site of each monomer is located at the center of the αβ-barrel. The gene for aldolase A (ALDOA) is located on chromosome 16q22–q24. It spans 7.5 kb and consists of 12 exons. Multiple transcription-initiation sites have been determined and ALDOA pre-mRNA is spliced in a tissue-specific manner.

Aldolase deficiency (OMIM 103850) is a very rare disorder and only six patients from five families have been described. Patients all displayed moderate chronic hemolytic anemia. Enzyme stability was decreased in a male patient described by Beutler et al. In addition to hemolytic anemia, this patient also displayed mental retardation and dysmorphic features. The two patients reported by Miwa et al. suffered from severe hemolytic anemia, exacerbated by infection, but none of the features described by Beutler et al. In one of these patients the causative mutation concerned a homozygous substitution of aspartic acid to glycine at residue 128. The patient reported by Kreuder et al. suffered from hemolytic anemia, myopathy, and psychomotor retardation. In this case, aldolase deficiency was caused by an homozygous ALDOA missense mutation that predicted a glutamic acid to lysine substitution at residue 206 at the subunit interface. Two additional patients were recently reported; clinical manifestations of the first included transfusion-dependent anemia but normal cognitive function, with death at age 4 associated with rhabdomyolysis. She was compound heterozygous for a null mutation, Arg303Stop, and a missense mutation, Cys338Tyr, in ALDOA. The second patient was a child with suspected hemolytic anemia and myopathic symptoms at birth in whom a
heterozygous Gly346Ser substitution was identified which affected the flexible C-terminal region of the enzyme.⁵⁰

**Triosephosphate Isomerase**

Triosephosphate isomerase (TPI) is the glycolytic enzyme with the highest activity in vitro.¹² TPI catalyzes the interconversion of glyceraldehyde-3-phosphate and DHAP (Figure 1). It consists of a dimer with two identical subunits of 248 amino acids (27 kDa).⁵² No TPI isozymes are known but three distinct electrophoretic forms can be distinguished as a result of post-translational modifications.⁵³ In red blood cells TPI activity is not maturation dependent. TPI is transcribed from a single gene (TPII) on chromosome 12p13 that consists of seven exons and spans 3.5 kb.⁵⁴ Three processed pseudogenes have been identified.⁵⁴

TPI deficiency (OMIM 190450) is a rare autosomal recessive disorder, characterized by hemolytic anemia at onset, often accompanied by neonatal hyperbilirubinemia requiring exchange transfusion. In addition, patients display progressive neurological dysfunction,⁵⁵ increased susceptibility to infection, and cardiomyopathy.⁵⁶ Patients show an 20- to 60-fold increased DHAP concentration in their erythrocytes,⁵⁷ consistent with a metabolic block at the TPI step. Most affected individuals die in childhood before the age of six years but there are remarkable exceptions. In a Hungarian family with TPI deficiency, two adult germ-line identical compound heterozygous brothers display strikingly different phenotypes. Both have the same severe decrease in TPI activity and congenital hemolytic anemia, but only one suffers from severe neurological disorder. Studies aimed at the pathogenesis of this differing phenotype point to functional differences in lipid environment influencing the enzyme activities involved in these phenotypic differences.⁵⁸
Fourteen mutations have been described in \textit{TPI1} and the most frequently occurring mutation (74\%) causes a glutamic acid to aspartic acid change at residue 104.\textsuperscript{59} Knowledge of the crystal structure of the human enzyme (PDB entry 1HTI) has provided insight into the probable effect of this mutation, which likely perturbs the local structure of the active site.\textsuperscript{52} The enzymatic activity of the Glu104Asp mutant is further reduced by its attachment to erythrocyte inside-out vesicles and to microtubules of brain cells.\textsuperscript{60} Haplotype analysis strongly suggested a single origin for the Glu104Asp substitution whereby the common ancestor originated from Northern Europe.\textsuperscript{61} This is in agreement with the European origin of almost all TPI-deficient individuals.

An animal model of TPI deficiency caused by missense and nonsense mutations has been developed in ethylnitrosourea-treated mice. Heterozygous animals display a 50\% loss of enzymatic activity whereas homozygosity for these TPI null alleles is lethal at an early stage of development.\textsuperscript{62}

\textit{Phosphoglycerate Kinase}

Phosphoglycerate kinase generates one molecule of ATP by catalyzing the reversible conversion of 1,3-bisphosphoglycerate to 3-phosphoglycerate (Figure 1). This reaction can be bypassed by the Rapoport–Luebering shunt (see “Rapoport–Luebering Shunt”), thus preventing the formation of the second ATP molecule. Two isozymes of PGK exist: PGK-1, ubiquitously expressed in all somatic cells, and PGK-2, expressed only in spermatozoa.\textsuperscript{63} PGK-1 is a 48-kDa monomer consisting of 417 amino acids.\textsuperscript{64} The three-dimensional structure of horse muscle PGK, highly homologous to the human enzyme, reveals that PGK consists of two domains. These domains are connected by a conserved hinge, allowing for conformational freedom.\textsuperscript{65} The
ADP/ATP binding site is located on the C-terminal domain while phosphoglycerates bind the N-terminal domain. A conformational rearrangement involving bending of the hinge occurs upon binding of both substrates, bringing them in position for phosphate transfer.66

The gene encoding PGK-1 (PGK1) is located on Xq13.3, thereby rendering PGK deficiency an X-linked disorder. PGK1 spans 23 kb and is composed of 11 exons.67

PGK deficiency (OMIM 311800) is characterized by chronic hemolytic anemia (often fully compensated), dysfunction of the central nervous system, and myopathy. However, the phenotype of PGK-1 deficiency is highly variable because patients usually do not display all three clinical features.68 PGK activity varies between 0 and 20% of normal but there is no correlation of residual enzymatic activity with clinical severity. Fourteen different mutations in PGK1 have been described in association with PGK deficiency.40 Most of these mutations are missense mutants of which one, in addition to encoding a non-conserved Glu252Ala substitution, resulted in aberrant splicing with a consequent 90% reduction in mRNA levels.69

**Pyruvate Kinase**

Pyruvate kinase catalyzes the irreversible phosphorylgroup transfer from phosphoenolpyruvate to ADP, yielding pyruvate and the second molecule of ATP in glycolysis (Figure 1). PK is a key regulatory enzyme of glycolysis, and pyruvate is crucial for several metabolic pathways. The enzyme is active as a tetramer, and four different isozymes are expressed in mammals. R-type PK expression is confined to the red blood cell70 whereas L-type PK is predominately expressed in the liver. The PK-R and PK-L subunits are both transcribed from a single gene (PKLR), located on chromosome 1q21, by the use of alternative promoters.70,71 PKLR consists of 12 exons
and spans 9.5 kb. Exon 1 is erythroid-specific whereas exon 2 is liver specific. Hence, exons 3 to 12 are included in both liver- and red blood cell-specific mRNAs, and encode a PK-R subunit of 574 amino acids whereas the PK-L subunit comprises 531 amino acids. PK isozymes PK-M1 and PK-M2 are produced from a single gene (PKM) by means of alternative splicing. PK-M1 is expressed in skeletal muscle, heart, and brain, and it is the only isozyme that is not subjected to allosteric regulation. The PK-M2 isozyme is expressed in early fetal tissues, but also in most adult tissues, including leucocytes and platelets.

In basophilic erythroblasts, both PK-R and PK-M2 are expressed. During further erythroid differentiation and maturation, a switch in isozymes occurs whereby progressively increased PK-R expression gradually replaces PK-M2. This is in part due to changes in protein synthesis and degradation rates. Human red blood cell PK consists of two distinct species, PK-R1 and PK-R2. PK-R1 predominates in reticulocytes and young erythrocytes, whereas mature red blood cells mainly possess PK-R2. PK-R1 is a homotetramer composed of four PK-R, also called L’, subunits (L’4). Limited proteolytic degradation of this 63 kDa PK-R subunit renders a 57-58 kDa PK-L subunit that is incorporated in the heterotetramer PK-R2 (L2L’2). The enzymatic activity of PK decreases with increasing cell age of the erythrocyte.

PK is allosterically activated by phosphoenolpyruvate and FBP, and negatively regulated by its product ATP. Furthermore, PK has an absolute requirement for cations, normally Mg2+ and K+. Recently, the three-dimensional structure of human erythrocyte PK (PDB entry 1LIU) was elucidated. Each PK-R subunit can be divided into four domains (Figure 3), the N domain (residues 1–84), A domain (residues 85–159 and 263–431), B domain (residues 160–262), and C domain (residues 432–574). Domain A is the most highly conserved whereas the B and C
The active site lies in a cleft between the A domain and the flexible B domain. The B domain is capable of rotating away from the A domain generating either the ‘open’ or ‘closed’ conformation. The C domain contains the binding site for FBP. The allosteric transition from the inactive T-state to the active R-state involves the simultaneous and concerted rotations of entire domains of each subunit, in such a way that all subunit and domain interfaces are modified. The allosteric and catalytic sites are able to communicate with each other across the relatively long distance that separates the FBP binding site from the catalytic center. The A/A’ and C/C’ subunit interface interactions and the A/B interdomain interactions are considered key determinants of the allosteric response.

PK deficiency (OMIM 266200) is the most common cause of nonspherocytic hemolytic anemia due to defective glycolysis and is inherited in an autosomal recessive manner. The estimated prevalence is 51 cases (i.e. homozygous or compound heterozygous patients) per million in the white population. Two major metabolic abnormalities result from PK deficiency: ATP depletion and increased 2,3-DPG content. However, the precise mechanisms that lead to a shortened lifespan of the mature PK-deficient erythrocyte are still unknown. The increased 2,3-DPG levels ameliorate the anemia by lowering the oxygen-affinity of hemoglobin (see “Rapoport–Luebering Shunt”). Phenotypically, the clinical picture varies from severe hemolysis causing neonatal death to a well compensated hemolytic anemia. Some PK-deficient patients present with hydrops fetalis. Reticulocytosis is almost always observed. Splenectomy often ameliorates the hemolysis, especially in severe cases, and increases reticulocyte counts even further. Persistent expression of the PK-M2 isozym has been reported in the red blood cells of patients (and animals, see...
below) with severe PK deficiency. The survival of these patients, though not in all cases, may be enabled by this compensatory increase in PK activity.

By the year 2005, more than 160 mutations in PKLR have been reported to be associated with pyruvate kinase deficiency. A schematic overview is presented in Figure 4. Most mutations (70%) are missense mutations affecting conserved residues in structurally and functionally important domains of PK. In the European and North-American population, the most frequently detected mutations are missense mutants c.1456C>T (Arg486Trp), c.1529G>A (Arg510Gln), c.994G>A (Gly332Ser), and nonsense mutant c.721G>T (Glu241Stop). There appears to be no direct relationship between the nature and location of the substituted amino acid and the type of molecular perturbation. As in TPI deficiency, patients with identical genotypes may be differently affected. For example, the clinical phenotype of twelve c.1529G>A homozygotes ranges from a very mild compensated hemolysis to a severe anemia. One transcriptional mutant is of particular interest because it silences erythroid-specific PKLR transcription completely. The causative single-base change disrupts a putative binding domain for an as yet unidentified trans-acting factor that mediates the effects of factors necessary for regulation of PK gene expression during red cell differentiation and maturation.

PK deficiency has also been recognized in dogs and mice. In both species, the deficiency causes severe anemia and marked reticulocytosis, closely resembling human PK deficiency. Basenji dogs lack PK-R enzymatic activity as a result of a frameshift mutation. Instead, only the PK-M2 isozyme is expressed in their red blood cells. PK-deficient mice homozygous for a missense mutation show delayed switching from PK-M2 to PK-R, resulting in delayed onset of the hemolytic anemia. Another loss of function mutation in homozygous PK-deficient mice underlies the
malaria resistance in these animals and may, similarly, protect humans against malaria.  

**Glyceraldehyde-3-phosphate Dehydrogenase, Monophosphoglycerate Mutase, Enolase, and Lactate Dehydrogenase**

Red blood cell deficiencies of glyceraldehyde-3-phosphate dehydrogenase and enolase have been described in association with hemolytic anemia but a causal relationship was not established.

Monophosphoglycerate mutase (MPGM) in erythrocytes is a homodimer, composed of two of the ubiquitously expressed B subunits. Only one case of B subunit MPGM deficiency is known to date: a patient with moderate hemolytic anemia due to hereditary spherocytosis displayed, in addition, a 50% reduction in red blood cell MPGM activity caused by a homozygous Met230Ile substitution. Deficiencies of either the H or M subunits of LDH have been reported. However, neither deficiency causes hemolytic anemia, despite strongly reduced enzymatic activity. Interestingly, homozygous LDH M subunit deficiency in mice is associated with chronic hemolytic anemia.

**Rapoport–Luebering Shunt**

In the erythrocyte, 2,3-DPG is synthesized and dephosphorylated in the Rapoport-Luebering Shunt (Figure 1). This glycolytic bypass is unique to mammalian red blood cells and represents an important physiological means to regulate oxygen affinity of hemoglobin. The oxygen affinity of hemoglobin is also influenced by slight
changes of blood pH, and a corresponding sensitivity for the pH exists in the Rapoport-Luebering Shunt that, again, permits changes in 2,3-DPG contents to fine-tune the oxygen affinity of hemoglobin. Quantitatively, 2,3-DPG is the major glycolytic intermediate in the red blood cell and its levels are about equal to the sum of the other glycolytic intermediates. Enzyme deficiencies proximal to the 2,3-DPG step (i.e. PGK and PK deficiency) show increased 2,3-DPG levels as a result of the respective metabolic blocks and of a retrograde accumulation of products of glycolysis. The increased 2,3-DPG levels result in a decreased oxygen affinity of hemoglobin so that oxygen is more readily transferred to tissue. Thus, the anemia is ameliorated and exertional tolerance is improved. This beneficial effect is absent in the distal glycolytic enzyme defects HK, GPI, PFK, aldolase, and TPI deficiency that all cause a decrease in 2,3-DPG levels. Several of the distal glycolytic enzymes (i.e. HK and PFK) are inhibited by 2,3-DPG.

Both reactions in the Rapoport-Luebering Shunt are catalyzed by the erythrocyte-specific multifunctional enzyme bisphosphoglycerate mutase (BPGM) which possesses synthase (formation of 2,3, BPG) and phosphatase (hydrolysis of 2,3-DPG to 3-phosphoglycerate) activity (Figure 1). BPGM is a homodimer, with 30-kDa subunits consisting of 258 amino acids. The enzyme is closely related to the glycolytic housekeeping enzyme MPGM. Recently, the crystal structure of human BPGM has been determined (PDB entry 1T8P), confirming the concept that BPGM and MPGM are structurally homologous enzymes, and providing a rationale for the specific residues that are crucial for synthase, mutase, and phosphatase activity.

The gene for BPGM (BPGM) has been mapped to chromosome 7q31-34 and it consists of three exons, spanning more than 22 kb. BPGM deficiency (OMIM 222800) is a very rare autosomal recessive disorder and only two affected families
have been described. In the first family, patients had severely reduced 2,3-DPG levels and increased ATP levels. They were clinically normal and displayed no hemolytic anemia. Instead, they presented with erythrocytosis that likely resulted from the reduced 2,3-DPG levels and, consequently, the increased oxygen affinity of hemoglobin. These patients were later found to be compound heterozygous for a single-nucleotide change in BPGM that predicted the replacement of a highly conserved arginine by cysteine at residue 89 (BPGM Créteil I) and a single-nucleotide deletion that introduces a premature stop codon and, consequently, encodes a truncated peptide (BPGM Créteil II). In the second BPGM-deficient family, the asymptomatic proband also presented with secondary erythrocytosis. He displayed markedly decreased BPGM enzyme activity levels whereas his consanguineous parents showed normal 2,3-DPG levels but BPGM activity levels of approximately 50% of normal. In addition, all family members had markedly decreased glucose-6-phosphate dehydrogenase activity although there was no laboratory evidence of hemolysis. DNA sequencing of the BPGM gene showed that the propositus was homozygous for a point mutation in exon 2 that predicted the substitution of Arg62 by Gln.

Concluding remarks

A variety of clinical features are associated with the described hereditary enzymopathies of the red blood cell. This is mainly due to the role of the affected enzyme in glycolysis as well as the underlying molecular alteration responsible for defective enzymatic function. Therefore, a better understanding of the clinical phenotype of defective glycolysis in the red blood cell requires knowledge regarding
the genetic, biochemical, and structural consequences of mutations in the genes and
the respective enzymes they encode. However, the phenotype is not solely dependent
on the molecular properties of mutant proteins but rather reflects a complex interplay
between physiological, environmental, and other (genetic) factors. Putative
phenotypic modifiers include differences in genetic background, concomitant
functional polymorphisms of other glycolytic enzymes (many enzymes are regulated
by their product or other metabolites), post-translational modification, epigenetic
modification, ineffective erythropoiesis, and different splenic function. Also, aberrant
enzymatic function in non-erythroid tissues may influence the clinical outcome of
hereditary enzymopathies. Hence, future research aimed at the relationship between
genotype and phenotype correlation in red blood cell enzymopathies will also have to
take these phenotypic modifiers into account.
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References


Figure Legends

Figure 1. Schematic overview of the Embden-Meyerhof Pathway and the Rapoport-Luebering shunt.

Figure 2. Human hexokinase in complex with glucose, phosphate, and ADP.
Ribbon representation of human hexokinase I with N-terminal and C-terminal halves depicted in red and blue, respectively. Selected ligands are shown in ball-and-stick representation and colored green (glucose), orange (ADP), and yellow (inorganic phosphate). The figure was generated from the atomic coordinates of the HK-I ADP/glucose complex (protein data bank entry 1DGK) using the program PyMOL (DeLano, W.L. The PyMOL Molecular Graphics System (2002) on World Wide Web http://www.pymol.org).

Figure 3. Human erythrocyte pyruvate kinase in complex with substrate analog phosphoglycolate and the allosteric activator fructose-1,6-diphosphate.
Ribbon representation of the human erythrocyte pyruvate kinase monomer (A) and tetramer (B). Phosphoglycolate and fructose-1,6-diphosphate are shown in ball-and-stick representation, and colored yellow and grey, respectively. Metal ions in the active site are shown as blue (Potassium) and pink (Manganese) spheres. (A) PK monomer with domains N, A, B, and C colored violet, cyan, orange, and lime, respectively. (B) PK tetramer with individual subunits colored lime, cyan, violet, and orange. The figures were generated from the atomic coordinates of protein data bank entry 1LIU using the program PyMOL (DeLano, W.L. The PyMOL Molecular Graphics System (2002) on World Wide Web http://www.pymol.org).
Figure 4. Distribution of *PKLR* mutations associated with PK deficiency.

Schematic representation of the *PKLR* gene and its erythroid-specific promoter. Exons, but not introns, are drawn to scale. Exons are numbered and depicted as grey rectangles with 5’- and 3’-noncoding sequences in black. The open rectangle represents the liver-specific exon 2. Nucleotides are numbered starting from the ATG in red blood cell-specific exon 1. The location of the more than 160 mutations associated with PK deficiency is indicated by vertical lines. Double-sized vertical lines represent multiple base changes at the same nucleotide position. The three (large) deletions known to date are indicated by horizontal lines.
Figure 1.
Figure 4.
The energy-less red blood cell is lost - erythrocyte enzyme abnormalities of glycolysis

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