

Leader

The molecular basis of disorders of red cell enzymes

Mary F McMullin

The mature red cell has no nucleus or organelles and therefore cannot synthesise protein or lipids. It is totally dependent on glycolysis to convert glucose into an energy source. Glucose is phosphorylated by hexokinase to glucose-6-phosphate. This is the substrate for anaerobic glycolysis which proceeds through the Embden-Meyerhof pathway to pyruvate with production of ATP (fig 1). Some of the glucose-6-phosphate may also be processed by oxidative glycolysis in the pentose phosphate pathway (fig 2). Around 20 enzymes facilitate these two major pathways and deficiency of any of these enzymes may cause haemolytic anaemia. The incidence of deficiency varies from over 400 million cases worldwide in glucose-6-phosphate dehydrogenase (G6PD) deficiency to single case report in some of the other enzymes. I shall discuss the molecular basis of some of these enzyme deficiencies in this review.

Deficiency of enzymes involved in the Embden-Meyerhof pathway**PYRUVATE KINASE DEFICIENCY**

Pyruvate kinase (PK) catalyses the conversion of phosphoenolpyruvate (PEP) to pyruvate. The enzyme exists as four isoenzymes. PK-L is expressed in the liver and PK-R in red cells. These two forms are encoded by a single gene, PK-LR, located at 1q21 but tissue specific promoters generate mRNAs with different 5' end sequences. PK-M₁ is the form in muscle and brain, and PK-M₂ is present in fetal and most adult cells including white blood cells and platelets. It is encoded by the PK-M gene located at 15q22 and the isoforms are produced by alternative splicing. During erythroid differentiation the PK-M₂ form is progressively replaced by PK-R.¹

In PK deficiency PK-R is functionally defective. PK deficiency presents as a nonspherocytic haemolytic anaemia which can be anything from mild compensated haemolysis to severe anaemia. Approximately 400 patients with the deficiency have been described and the inheritance is autosomal recessive. Nearly 100 gene mutations have been identified.²⁻⁶ The majority are missense mutations but splicing mutations, insertions, and deletions also occur. The mutations are widely distributed throughout the gene. Although most mutations occur only once, some appear relatively often;

for example a missense mutation in codon 510 (CGA;Arg → CAA;Gln) is reported in 30% of cases in Northern European and United States populations. Despite patients having the same genetic abnormality there can be wide variation in the clinical expression of the disease. Thus other genetic or environmental factors influence the phenotype.⁵ Molecular diagnosis is now nearly routine and carrier detection and prenatal diagnosis is feasible.

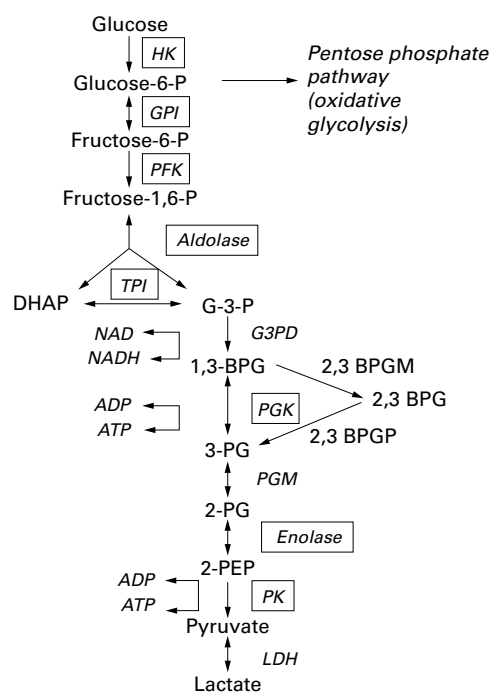


Figure 1 The Embden-Meyerhof pathway for anaerobic glycolysis in the erythrocyte. Boxed enzymes cause haemolytic disease when deficient. ADP, adenosine diphosphate; ATP, adenosine triphosphate; DHAP, dihydroxyacetone phosphate; G-3-P, glyceraldehyde-3-phosphate; G3PD, glyceraldehyde-3-phosphate dehydrogenase; GPI, glucose phosphate isomerase; HK, hexokinase; LDH, lactate dehydrogenase; NAD, nicotinamide adenine dinucleotide; NADH, reduced NAD; PFK, phosphofructokinase; PGK, phosphoglycerate kinase; PGM, phosphoglycerate mutase; PK, pyruvate kinase; TPI, triose phosphate isomerase; 1,3-BPG, 1,3-bisphosphoglycerate; 2-PG, 2-phosphoglycerate; 2-PEP, 2-phosphoenolpyruvate; 2,3-BPG, 2,3-bisphosphoglycerate; 2,3-BPGM, 2,3-bisphosphoglycerate mutase; 3-PG, 3-phosphoglycerate.

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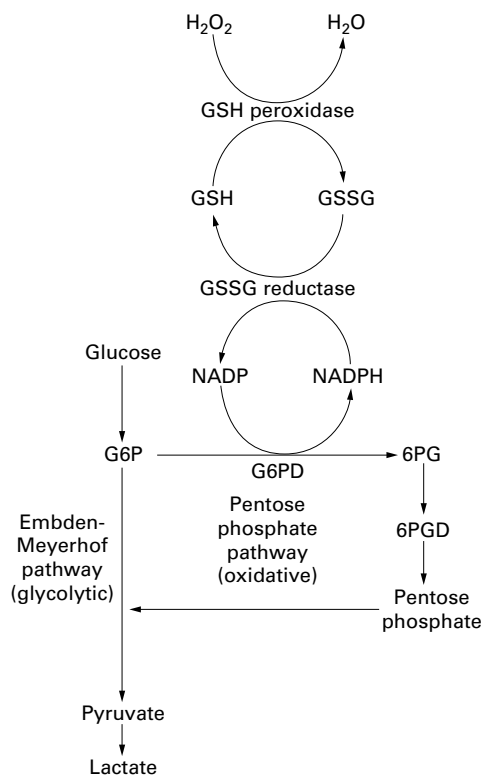


Figure 2 The pentose phosphate pathway for red cell oxidative glycolysis. G6P, glucose-6-phosphate; G6PD, glucose 6-phosphate dehydrogenase; GSH, glutathione; GSSG, oxidised glutathione; H₂O₂, hydrogen peroxide; NADP, nicotinamide adenine dinucleotide phosphate; 6PG, 6-phosphogluconolactone; 6PGD, 6-phosphogluconolactone dehydrogenase.

HEXOKINASE DEFICIENCY

Hexokinase catalyses the first step in the Embden-Meyerhof pathway, the conversion of glucose to glucose-6-phosphate. The enzyme is located at 10p11.2. Approximately 20 cases of hexokinase deficiency have been described and the patients present with non-spherocytic haemolytic anaemia. It is an autosomal recessive disorder. The molecular defect has been described in at least one patient in whom there was a point mutation in one allele of the hexokinase gene and a deletion in the other allele producing clinical disease.⁷

GLUCOSE PHOSPHATE ISOMERASE DEFICIENCY

Glucose phosphate isomerase (GPI) catalyses the conversion of glucose-6-phosphate to fructose-6-phosphate. The GPI gene is located at 19cen-q13. Over 45 cases of GPI deficiency have been described, generally presenting with compensated haemolytic anaemia but in one case associated with hydrops fetalis and in two cases associated with mental retardation. The inheritance is autosomal recessive and cases are compound heterozygotes or homozygotes. Approximately 20 mutations have been described which are distributed throughout the gene.^{8,9} These are missense mutations, deletions, and nonsense mutations. GPI is identical to neuroleptin, a neurotrophic factor for spinal and sensory neurones, and may have a function outside carbohydrate metabolism.

PHOSPHOFRUCTOKINASE DEFICIENCY

Phosphofructokinase (PFK) catalyses the conversion of fructose-6-phosphate to fructose 1-6 phosphate. Over 35 cases of PFK deficiency have been reported. There are different genes for the different PFK proteins in muscle (PFK-M), liver (PFK-L), and platelets (PFK-P). It is a multisystem disease, the expression of which varies owing to the variable expression in the tissues. Manifestations include haemolytic anaemia and myopathy. The commonest form is Tarui's disease or glycogen storage disease type VII. At least 11 mutations of the PFK-M gene, which is located at 1cen-q32, have been described—splicing defects, nucleotide deletions, and point mutations.¹

TRIOSEPHOSPHATE ISOMERASE DEFICIENCY

Triosephosphate isomerase (TPI) catalyses the interconversion of dihydroxyacetone phosphate and glyceraldehyde-3-phosphate. Over 35 cases of TPI deficiency have been reported. It presents as a multisystem disorder with progressive neurological dysfunction, cardiomyopathy, and increased susceptibility to infection. Affected individuals die in early childhood. The inheritance is autosomal recessive but it is interesting, given the rarity of the disorder, that 5% of African Americans are heterozygotes. The gene is encoded at 12p13. Although mutations have been described in single families, the majority of cases are due to a missense mutation, GAG;Glu → GAC;Asp, in codon 104, in contrast to the genetic heterogeneity of other disorders.¹⁰ There is evidence for a single origin for this mutation in a single ancestor.¹¹

PHOSPHOGLYCERATE KINASE DEFICIENCY

Phosphoglycerate kinase (PGK) catalyses the interconversion of 1,3 diphosphoglycerate and 3-phosphoglycerate. Approximately 20 cases of PGK deficiency have been described. Inheritance is X linked, the gene being located at Xq13. Affected males usually present with haemolytic anaemia and neurological abnormalities, including mental retardation and myopathy. Females have mild haemolytic anaemia owing to the lyonisation of the X chromosome. At least nine mutations have been described, each resulting in a unique point mutation.¹²

OTHER ENZYME DEFICIENCIES

Deficiencies of the enzymes aldolase, enolase, 2,3-bisphosphoglycerate mutase (2,3-BPGM), and lactate dehydrogenase (LDH) have all been reported in isolated cases, and haemolysis has been described as part of the deficiency. Molecular abnormalities have now been described in all of these deficiencies.¹

Abnormalities of erythrocyte nucleotide metabolism

Nucleotide metabolic pathways are essential for maintenance of the energy pool of red cells. Pyrimidine 5' nucleotidase (P5'N), adenosine deaminase, and adenylate kinase are vital to nucleotide cycling, and abnormalities of these enzymes are associated with decreased red cell

survival. P5'N is the most common of these disorders but the precise molecular defect has not been clarified because the gene has not been identified.

Adenosine deaminase deficiency has been detected in red cells as part of autosomal recessive severe combined immunodeficiency. Adenylate kinase deficiency has also rarely been described as a cause of haemolytic anaemia and in some cases mental retardation.¹³ The molecular defect is not yet clear.

Deficiency of enzymes involved in the pentose phosphate pathway

GLUCOSE-6-PHOSPHATE DEHYDROGENASE

By far the most common abnormality occurring in the pentose phosphate pathway is a defect in glucose-6-phosphate dehydrogenase (G6PD). This enzyme oxidizes glucose-6-phosphate (G6P) to 6-phosphogluconolactone (6PG) with concomitant reduction of nicotinamide adenine dinucleotide phosphate (NADP) to the reduced form, NADPH. (fig 2) In the red cell this is the only source of NADPH which maintains glutathione (GSH) in the reduced state. GSH in turn reduces peroxides and other reactive oxygen species. G6PD is therefore essential to protect the red cell from oxidative damage.

The G6PD gene is located on the long arm of the X chromosome (Xq28). It consists of 13 exons which encode a 515 amino acid protein with a molecular weight of 59 kDa.

The active form of the enzyme exists as a dimer or tetramer and tightly binds NADP. G6PD deficiency affects over 400 million people worldwide. In most cases it exists as a balanced polymorphism, affected individuals having the advantage of resistance to malaria. These people are asymptomatic unless exposed to an agent which precipitates an episode of acute haemolysis.¹⁴ A small subset of cases, usually with more severe chronic haemolysis, occurs sporadically worldwide.

The vast majority of abnormalities in the G6PD gene are point mutations causing single amino acid substitutions. This contrasts with many other genetic disorders and implies that only disorders with some residual G6PD activity exist. Five small deletions have been described in which the number of bases deleted is a multiple of 3, leading to the deletion of a few amino acids and residual enzymic activity. The one stop codon mutation reported which results in a truncated protein of 427 amino acids exists only in the heterozygous state, as has the one splice site mutation which has been found to have residual enzymic activity.^{15 16} It seems probable that total absence of G6PD activity would be incompatible with life.

In some populations the gene frequency of G6PD deficiency is very high, but very few mutations may exist in individual populations. Thus some of these mutations may have a single origin. Despite the fact that G6PD deficiency was initially characterised biochemically and variants from different areas were given individual names, molecular analysis has shown that many of the so called variants are the same.

The situation with the sporadic variety is different. The same mutation appears to have arisen independently in cases in different areas.¹⁷ This suggests that mutations capable of causing chronic haemolysis are restricted to a small number of changes that can produce an enzyme with low red cell activity but considerable activity in other tissues.

Three dimensional models of human G6PD have allowed some insight into how mutations can cause clinical disease.¹⁶ In the sporadic form many of the mutations are situated at the dimer interface and affect NADP binding. Binding NADP causes increased stability of the dimeric form, and mutations affecting dimer formation or stability have a dramatic effect on NADP binding.

The polymorphic mutations are not associated with any one area of the tertiary structure. However, G6PDA mutations result in loss of folding of the protein and this form may have a shorter half life and account for the disease phenotype. Further research in this area may elucidate the relation between mutations and the presenting disease.

OTHER ENZYME DEFICIENCIES

Two enzymes are required for glutathione (GSH) synthesis, γ -glutamyl synthetase and GSH synthetase, and reduced activity of these enzymes has been associated with mild haemolysis with and without other more generalised abnormalities in isolated cases.^{18 19} The molecular defect in individual cases has not been isolated.

Glutathione reductase and glutathione peroxidase levels have been reported as decreased in various nutritional deficiencies and in isolated case reports. No definite link to haemolysis has been established. Likewise 6-phosphogluconate dehydrogenase (6PGD) deficiency has been well documented but has no significance for red cell survival.

Conclusion

It is evident that functionally deficient enzymes can cause characteristic disorders of the red cell, although many such enzymes have a more widespread occurrence in the body. Molecular analysis of many of these defects has now become semi-routine and opens avenues for prenatal diagnosis for the first time and the hope of cure with gene therapy.

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