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Characterization of the first described mutation of human red blood cell phosphoglycerate mutase

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Abstract

In a patient with clinical diagnosis of Hereditary Spherocytosis and partial deficiency (50%) of red blood cell phosphoglycerate mutase (PGAM) activity, we have recently reported [A. Repiso, P. Pérez de la Ossa, X. Avilés, B. Oliva, J. Juncá, R. Oliva, E. Garcia, J.L.L. Vives-Corrons, J. Carreras, F. Climent, Red blood cell phosphoglycerate mutase. Description of the first human BB isoenzyme mutation, Haematologica 88 (2003) (03) ECR07] the first described mutation of type B PGAM subunit that as a dimer constitutes the PGAM (EC 5.4.2.1) isoenzyme present in red blood cells. The mutation is the substitution c.690G>A (p.Met230Ile). In this report, we show that the mutated PGAM possesses an abnormal behaviour on ion-exchange chromatography and is more thermo-labile that the native enzyme. We also confirm that, similar to the PGAM isoenzymes from other sources, the BB-PGAM from human erythrocytes has a ping pong or phosphoenzyme mechanism, and that the mutation does not significantly change the K_m and K_i values, and the optimum pH of the enzyme. The increased instability of the mutated enzyme can account for the decreased PGAM activity in patient's red blood cells. However, the implication of a change of the k_{cat} produced by the mutation cannot be discarded, since we could not determine the k_{cat} value of the mutated PGAM.

Keywords: Phosphoglycerate mutase; Erythrocyte; Mutation; Kinetic property; Heat stability; Modellation

1. Introduction

Phosphoglycerate mutases are a family of enzymes essential in the metabolism of glucose and 2,3-bisphosphoglycerate (2,3-BPGA), which catalyze reactions involving the transfer of phospho groups among the three carbon atoms of phosphoglycerates. There are at least four types of phosphoglycerate mutases [1]: 2,3-BPGA-independent monophosphoglycerate mutase (PGAM), 2,3-BPGA-independent-Mn^{2+}-dependent PGAM, 2,3-BPGA-dependent PGAM and bisphosphoglycerate mutase (BPGAM). Only the last two types are present in mammals. The 2,3-BPGA-dependent PGAM (EC 5.4.2.1, formerly listed as EC 2.7.5.3) catalyzes the interconversion of 3-phosphoglycerate (3-PGA) and 2-phosphoglycerate (2-PGA) in the presence of the cofactor 2,3-BPGA. In addition, it also catalyzes the synthesis of 2,3-BPGA (1,3-BPGA+3-PGA⇌3-PGA+2,3-BPGA) and its breakdown (2,3-BPGA→3-PGA+Pi). However, these two reactions occur at much lower rates than the interconversion of the monophosphoglycerates. The BPGAM (EC 5.4.2.4./EC 3.1.3.13, formerly listed as EC 2.7.5.4) is frequently named bisphosphoglycerate synthase. It catalyzes as main reactions the synthesis and the breakdown of 2,3-BPGA, although it also possesses a high level of monophosphoglycerate mutase activity [1].

In mammalian tissues, three PGAM isoenzymes exist, which result from the homo- and heterodimeric combina-

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tions of two subunits, M and B, encoded by two different genes. In adult mammals, skeletal muscle and mature sperm cells contain almost exclusively type MM-PGAM, whereas type BB-PGAM is found in most other tissues and in erythrocytes. Type MB-PGAM is present only in heart, which also contains MM-PGAM and BB-PGAM isoenzymes. The three PGAM isoenzymes have very similar kinetic properties, but differ in their susceptibility to inactivation by Hg^{2+} and sulfhydryl group reagents, and in their thermal lability. In mammals, BPGAM is a homodimer constituted by a subunit encoded by a gene closely related to the PGAM genes. In addition to the BPGAM homodimer, mammalian tissues possess hybrids of type BPGAM subunit with types M and B-PGAM subunits. The homodimer is found in all tissues, although it is particularly abundant in erythrocytes. The BPGAM/M-PGAM heterodimer is present in adult skeletal and cardiac muscle, and the BPGAM/B-PGAM heterodimer is in the brain, liver, kidney and erythrocytes. The two heterodimers have catalytic properties similar to those of the BPGAM homodimer [1].

Since 1964, several cases of BPGAM deficiency have been described in human erythrocytes [2], but only one has been characterized at genetic level [3–5], and since 1981 [6], several cases of type MM-PGAM deficiency in skeletal muscle have been reported, although only four different mutations have been detected [7–10]. Recently, we have reported [11] the first described mutation of type BB-PGAM isoenzyme in a patient with decreased PGAM activity in red blood cells. The patient was a 34-year-old woman with moderate normocytic anaemia and markedly increased reticulocyte count. Haemolytic tests performed demonstrated a negative direct antiglobulin test with markedly decreased red blood cell osmotic fragility, and the clinical diagnosis of Hereditary Spherocytosis was made. A battery of 18 red blood cell enzyme activity was also performed and a partial deficiency (50%) of normal PGAM activity was found. The other enzyme activities were normal or slightly increased in accordance with the increased number of reticulocytes. By RT-PCR and sequencing analysis, we detected a point mutation (c.690G>A) that causes the substitution (p.Met230Ile). In this work, we compare the catalytic and some other properties of the native human type BB-PGAM with those of the mutated enzyme.

2. Materials and methods

2.1. Materials

Purified phosphoglycerate mutase, enolase, pyruvate kinase and lactate dehydrogenase, substrates and cofactors were purchased from either Boehringer (Mannheim, Germany) or Sigma (St Louis, MO). β-Mercaptoethanol was from Merck (Darmstadt, Germany). Bovine serum albumin, microcrystalline cellulose (Sigmacell type 50) and α-cellulose fiber were from Sigma. Hydroxyapatite (Bio-Gel HTP) and Dowex AG-1-X8 were from BioRad (Hercules, CA), DEAE-Sephacel was from Amersham Pharmacia Biotech (Rainham, UK), and DEAE Cellulose (DE-23, fibrous anion exchanger) was from Whatman Bio Systems Ltd (Kent, UK). All other chemicals were reagent grade. 3-PGA free of 2,3-BPGA was prepared from the barium salt by purification on Dowex AG-1-X8 [12].

2.2. Enzyme assays and protein determination

The monophosphoglycerate mutase activity was assayed by coupling the formation of 2-PGA from 3-PGA with the enolase, pyruvate kinase and lactate dehydrogenase reactions [13]. The assay mixture contained, in a total volume of 1 ml in a 1-cm light path, cell equilibrated at 30 °C: 50 mM Tris–HCl buffer pH 7.4, 0.5 mM EDTA, 50 mM KCl, 10 mM $MgCl_2$, 2.5 mM ADP, 0.12 mM NADH, 5 mM 3-PGA, 0.25 mM 2,3-BPGA, 0.3 U of enolase, 0.15 U of pyruvate kinase and 0.5 U of lactate dehydrogenase. When thermal equilibration had been attained, the sample was added to the assay mixture and the decrease in A_{340} was recorded.

The 2,3-BPGA phosphatase activity was assayed by coupling the formation of 3-PGA from 2,3-BPGA with the phosphoglycerate mutase, enolase, pyruvate kinase and lactate dehydrogenase reactions [14]. The assay mixture contained, in a total volume of 1 ml in a 1-cm light path, cell equilibrated at 30 °C: 50 mM Tris–HCl buffer pH 7.4, 0.5 mM EDTA, 50 mM KCl, 10 mM $MgCl_2$, 2.5 mM ADP, 2 mM 2-phosphoglycolate, 0.12 mM NADH, 0.5 mM 2,3-BPGA, 0.04 U of PGAM, 0.3 U of enolase, 0.15 U of pyruvate kinase and 0.5 U of lactate dehydrogenase. When thermal equilibration had been attained, the sample was added to the assay mixture and the decrease in A_{340} was measured. The PGAM used as a coupling enzyme has 2,3-BPGA phosphatase activity, which is activated by 2-phosphoglycolate, but was negligible as compared with that of the samples assayed.

Protein was estimated by the method of Bradford [15], using the Bio-Rad Protein Assay Kit II and bovine serum albumin as a standard.

2.3. Determination of the kinetic constants

For the determination of the kinetic constants, the initial rates of conversion of 3-PGA to 2-PGA were measured by the NADH-coupled assay described above. The assay mixture contained, in a total volume of 1 ml in a 1-cm light path, cell equilibrated at 30 °C: 16.6 mM Tris–HCl buffer pH 7.4, 2.25 mM free Mg^{2+}, 0.33 mM ADP, 0.14 mM NADH, 0.02–2 mM 3-PGA, 0.33-50 μM 2,3-BPGA, 0.33 U of enolase, 0.25 U of pyruvate kinase and 1.5 U of lactate dehydrogenase. The free Mg^{2+} was maintained constant by the addition of $MgCl_2$ assuming binding constants of 255 mM^{-1} for 3-PGA and 111 mM^{-1} for 2,3-BPGA [16]. KCl was added to maintain constant the ionic strength to 0.08. When thermal equilibrium had been