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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

attained, 7 mU of PGAM in 80–90 μl were added to the assay mixture and the decrease in A_{340} was recorded.

The influence of pH on the initial rates was determined by the standard NADH-coupled assay, using a set of different buffers of the same ionic strength (0.2): 33 mM acetic–acetate buffer (pH 4.7, 5.2 and 5.8), 33 mM imidazole–HCl buffer (pH 6.5 and 7.0), 33 mM Tris–HCl buffer (pH 7.3, 7.9 and 8.5) [17].

2.4. Heat stability

For the determination of the thermal stability, the PGAM was incubated at 37 °C, 45 °C and 60 °C in 28 mM phosphate buffer pH 6.8, containing 1 mM EDTA, 1 mM β-mercaptoethanol and 1% bovine serum albumin, in the absence and in the presence of 2 mM 2,3-BPGA. The enzyme activity was measured at intervals under standard conditions (pH 7.2).

2.5. Purification of PGAM

Venous blood samples from the patient and two normal controls were collected with heparin as anticoagulant. After removing leukocytes and platelets by filtration through a cellulose/α-cellulose column, the hemolysates were obtained in 7.5 mM NaCl containing 2.4 mM EDTA and 0.6 mM β-mercaptoethanol as described by Beutler [13], frozen and stored at −80 °C. The hemolysates were freed of haemoglobin essentially as described by Harkness and co-workers [18,19]. After thawing and centrifugation to discard insoluble material, Tris–HCl buffer pH 7.5 was added to a 1.25 mM final concentration. DEAE-Cellulose was added to the hemolysates (0.5 g/10–20 ml) and the suspension was stirred for 4 h at 5 °C. The cellulose with adsorbed enzymes was washed free of haemoglobin on a Büchner filter with 10 mM Tris–HCl buffer pH 7.5, containing 1 mM EDTA and 1 mM β-mercaptoethanol (buffer A), and then eluted by stirring twice with 3 ml of the same mixture containing 250 mM KCl. The protein in the haemoglobin-free combined eluate was precipitated by 2 volumes of acetone (−20 °C). The precipitate was allowed to settle overnight at −20 °C, the clear supernatant decanted and the precipitate collected by centrifugation.

To separate PGAM from BPGAM and the hybrid BPGAM/B-PGAM, DEAE-Sephacel chromatography [20,21] and chromatography on hydroxyapatite [14,18–20,22–25] were used. For separation on DEAE-Sephacel, the acetone precipitate was dissolved in 1 ml of buffer A and, after centrifugation to discard insoluble material, a sample (0.8 ml, 12.5–18 mg protein/ml) was applied to a DEAE-Sephacel column (0.7×6.5 cm) equilibrated with the same buffer. After the column had been washed, it was eluted first with 50 ml of buffer A containing 70 mM KCl and then with 50 ml of the same buffer containing 90 mM KCl. At a flow rate of 0.5 ml/min, 0.5 ml fractions were collected and mixed with 0.5 ml of a 2% solution of bovine serum albumin in starting buffer. The collected fractions were assayed for monophosphoglycerate mutase activity (predominant in the PGAM enzyme) and for the 2,3-BPGA phosphatase activity (predominant in the BPGAM and in the hybrid BPGAM/B-PGAM enzymes).

For separation on hydroxyapatite, the acetone precipitate was dissolved in 1 ml of 10 mM potassium phosphate buffer pH 6.8, containing 1 mM EDTA and 1 mM β–mercaptoethanol (buffer B) and, after centrifugation, a sample (0.8 ml, 2.5–5 mg protein/ml) was applied to a hydroxyapatite column (0.7×6.5 cm) equilibrated with the same buffer. After thorough washing, the column was eluted with a linear gradient consisting of 25 ml of buffer B and 25 ml of 150 mM potassium phosphate buffer pH 6.8, containing 1 mM EDTA and 1 mM β-mercaptoethanol (buffer C). Fractions were collected as assayed as in the DEAE-Sephacel chromatography.

2.6. PGAM modelling

The structure of the native human type B PGAM subunit was built with the program MODELLER [26] using the constraints obtained from the sequence alignment to *E. coli* PGAM [27]. The structure of the mutated form of the subunit was obtained substituting Met230 by Ile with the program TURBO-FRODO [28].

3. Results and discussion

3.1. Isolation of PGAM

Figs. 1 and 2 reproduce the elution profiles obtained when the haemoglobin-free hemolysates from control blood and patient's blood, after acetone precipitation, were chromatographed on a DEAE-Sephacel and on a hydroxyapatite column, respectively. As shown in Fig. 1 panel "a", on DEAE-Sephacel chromatography, the monophosphoglycerate mutase and the 2,3-BPGA phosphatase activities from control hemolysates appeared as two separate peaks, eluted with 70 mM KCl and with 90 mM KCl, respectively, that correspond to PGAM and to BPGAM and the hybrid BPGAM/B-PGAM enzymes [20,21]. In contrast, the activities from the patient's hemolysates (Fig. 1 panel "b") appeared as a single peak eluted with 90 mM KCl. No monophosphoglycerate mutase activity was eluted with 70 mM KCl.

As shown in Fig. 2, on hydroxyapatite chromatography, a similar elution profile was obtained from the control (panel "a") and the patient's (panel "b") hemolysates. The monophosphoglycerate mutase activity was eluted as a single peak and the 2,3-BPGA phosphatase activity was eluted in three peaks, which correspond to the BB-PGAM, the BPGAM/B-PGAM hybrid and the BPGAM, respectively [21].

It has to be noted that, because of its very low values, the measurement of the monophosphoglycerate mutase activity

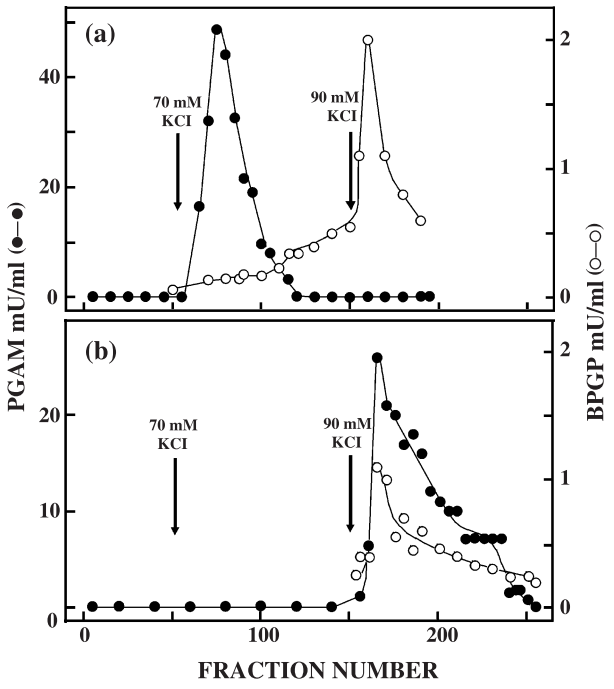

Fig. 1. Separation of the enzymes with monophosphoglycerate mutase (PGAM) and 2,3-BPGA phosphatase (BPGP) activities from control (panel "a") and patient's (panel "b") hemolysates by DEAE-Sephacel chromatography. Samples of 0.8 ml of control (18 mg protein/ml) and of patient's (12.5 mg protein/ml) hemolysates were applied to the column and eluted as described in the text.

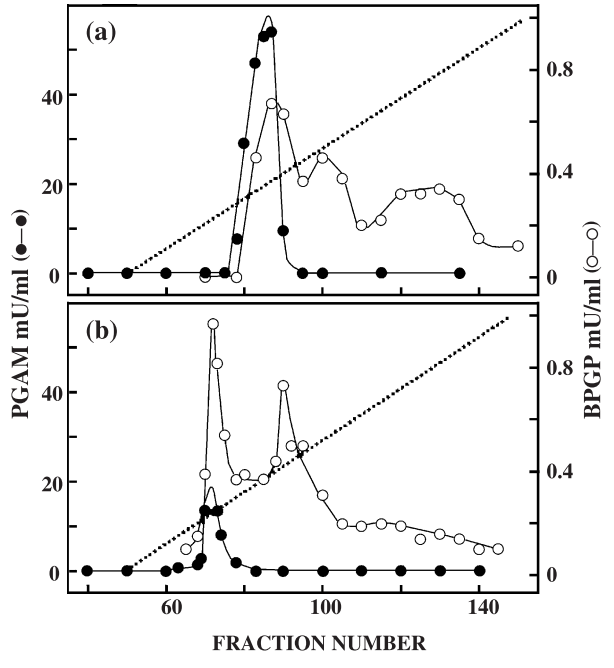

Fig. 2. Separation of the enzymes with monophosphoglycerate mutase (PGAM) and 2,3-BPGA phosphatase (BPGP) activities from control (panel "a") and patient's (panel "b") hemolysates by Hydroxyapatite chromatography. Samples of 0.8 ml of control (2.5 mg protein/ml) and of patient's (5 mg protein/ml) hemolysates were applied to the column and eluted as described in the text. (. . . . .) potassium phosphate gradient (10–150 mM).

in the peaks corresponding to the BPGAM/B-PGAM hybrid and to the BPGAM requires an incubation time much longer than that routinely used to assay the fractions collected from the columns.

It is concluded that the mutation (Met230Ile) causes a change in the chromatographic behaviour of type BB-PGAM and of the hybrid BPGAM/B-PGAM on DEAE-Sephacel columns. Although the substitution of Met by Ile does not involve any difference in the total charge of the PGAM subunit, the enzymes that possess the mutated form bind more strongly to the ion-exchange matrix. This suggests that, in addition to the ion-exchange effect, other type of binding occurs, which is affected by the Met230Ile mutation.

3.2. Thermal stability

As shown in Fig. 3, when incubated at 45 °C, the PGAM from controls (panel "a") was highly stable. In contrast, the mutated PGAM (panel "b") was 50% inactivated after 20 min of incubation and lost about 90% of its activity after 1 h.

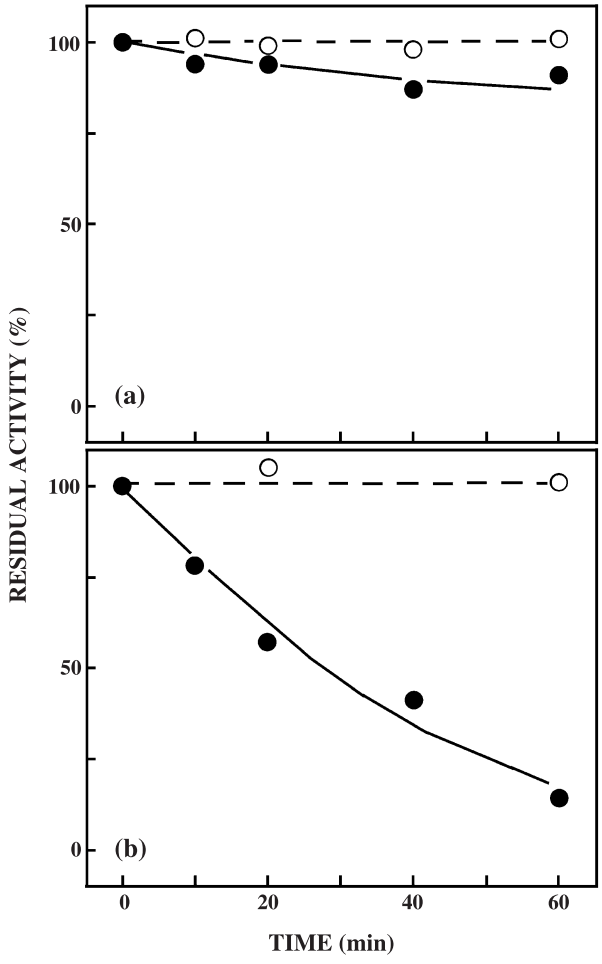

Fig. 3. Rates of heat inactivation of native (panel "a") and mutated (panel "b") PGAM at 45 °C. Incubations and assays were as described in the text. (●) incubations without 2,3-BPGA; (○) incubations with 2 mM 2,3-BPGA.

Table 1
Thermal stability of native and mutated BB-PGAM

	37 °C				60 °C	
	(1 h)		(24 h)		(1 h)	
2 mM 2, 3-BPGA	–	+	–	+	–	+
Native[a]	105 (0.10)	104 (0.06)	94 (0.17)	107 (0.06)	0	33 (0.15)
Mutated	87	96	37	88	0	5

[a] Mean value of two controls. Values are percentage with respect the initial value. Numbers inside parentheses are the absolute differences of the two values divided by the mean.

However, both the native and the mutated PGAM were fully protected by the cofactor 2,3-BPGA against thermal inactivation. As summarized in Table 1, after 24-h incubation at 37 °C, the native PGAM retained 94% of its activity, whereas the mutated PGAM was 63% inactivated. At this temperature, 2,3-BPGA also protected the enzymes against inactivation, although the protective effect was lower in the case of the mutated PGAM. After 1-h incubation at 60 °C, both the native and the mutated PGAM were fully inactivated, but, in contrast to the mutated enzyme, the native PGAM was partly protected by 2,3-BPGA.

These results indicate that the (Met230Ile) mutation decreases the thermal stability of PGAM and the protective effect of the cofactor 2,3-BPGA against thermal inactivation [12,29]. We show that the binding of 2,3-BPG to the PGAM active site is able to counteract the increased heat lability caused by the mutation at 45 °C, but not at a higher temperature. Therefore, it can be concluded that the decreased monophosphoglycerate mutase activity in the propositus erythrocytes can be at least partly explained by the effect of the mutation on the stability of the enzyme.

3.3. Kinetic pattern

The earlier attempts to elucidate the mechanism of PGAM by conventional initial velocity measurements led to conflicting results [30,31]. Later, it was shown by isotopic-induced transport tests based on flux kinetics and by isolation of the phosphoenzyme that the yeast, rabbit muscle and pig kidney PGAM possess a ping pong mechanism with a phosphoenzyme intermediate that involves a hystidyl residue [1]. Further kinetic data in agreement with the ping pong mechanism were reported for chicken breast muscle PGAM [32] and for types MM, MB and BB-PGAM isoenzymes purified from pig heart [29]. The crystal structure of the tetrameric PGAM from *S. cerevisiae* was first published in 1974 [33], and structures of different crystal forms, inhibitors and substrate complexes followed [34–38]. More recently, the crystal structures of the phosphohistidine-activated form of the dimeric PGAM from *E. coli* and of the vanadate–*E. coli* PGAM complex have been obtained, and have been shown structural changes on histidine phosphorylation that are significant in the catalytic mechanism [37–39].

The initial velocity studies reported on erythrocyte PGAM have also been conflicting. Chiba et al. [40,41] found a converging kinetic pattern for pig and human erythrocyte PGAM, but Stankiewicz and Hass [42] obtained parallel rather than converging lines for the human erythrocyte enzyme. Figs. 4 and 5 represent the effects of substrate and coenzyme concentrations on the activity of native BB-PGAM isolated from human erythrocytes by DEAE-Sephacel chromatography over a range of 0.02 mM to 2 mM 3-PGA and of 0.33 μM to 50 μM 2,3-BPGA. Fig. 4 shows the Lineweaver–Burk plots of $1/V$ vs. 1/[3-PGA] at different 2,3-BPGA concentrations, and Fig. 5 the plots of $1/V$ vs. 1/[2,3-BPGA] at different 3-PGA concentrations. As shown, at low concentrations, a series of parallel lines were obtained in both plots. Higher concentrations of substrate and cofactor produced competitive inhibition. This is the result expected for a ping pong mechanism provided that the breakdown of the phosphoenzyme is not appreciable when compared with the rate of dissociation of 2,3-BPGA [16,43]. As previously discussed [16], the converging line initial velocity pattern reported by Chiba and co-workers could result from the instability of the intermediate phosphoenzyme under their experimental conditions. There is also the possibility that in these experiments, there may

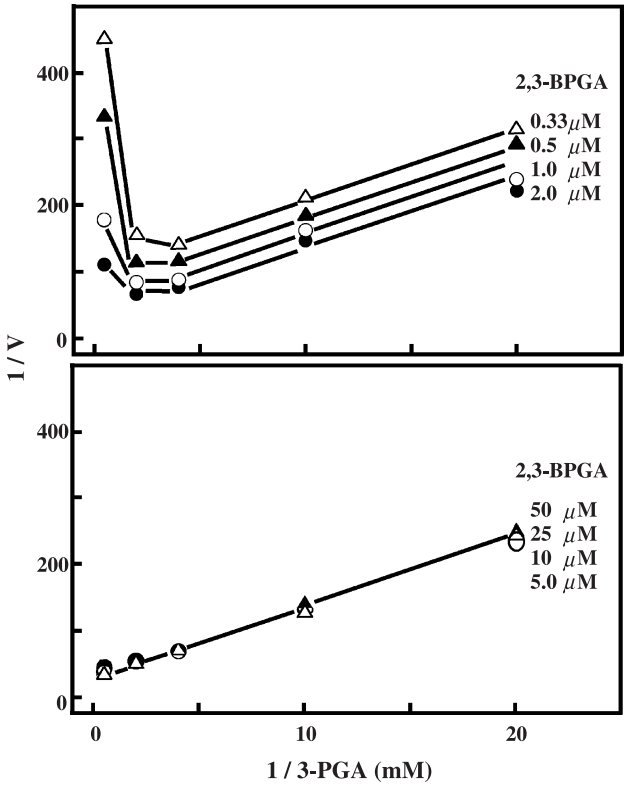

Fig. 4. Kinetic pattern of BB-PGAM from normal erythrocytes. Lineweaver–Burk plots of $1/V$ vs. 1/[3-PGA] at different 2,3-BPGA concentrations. Monophosphoglycerate mutase activity was assayed as described in the text.

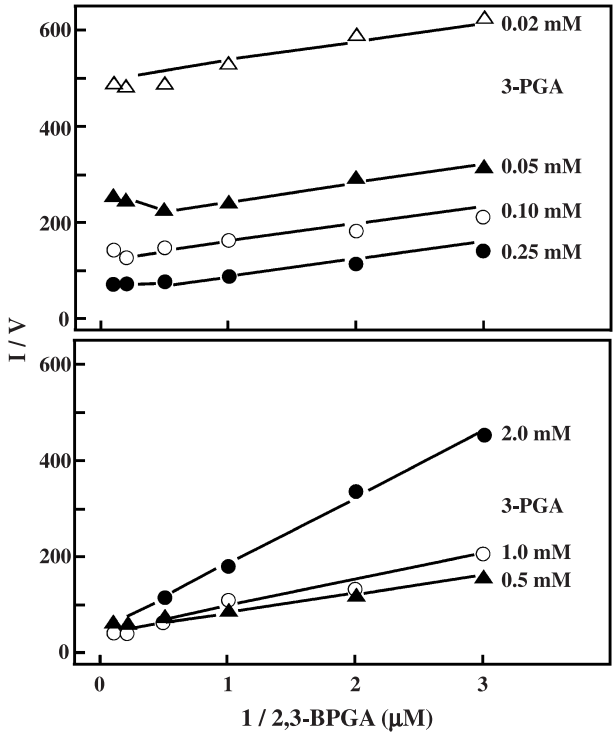

Fig. 5. Kinetic pattern of BB-PGAM from normal erythrocytes. Lineweaver–Burk plots of $1/V$ vs. 1/[2,3-BPGA] at different 3-PGA concentrations. Monophosphoglycerate mutase activity was assayed as described in the text.

have been a tendency to underestimate the initial velocities when both the 3-PGA and 2,3-BPGA concentrations were low. Under these conditions, significant product inhibition may have occurred, and relatively small errors in determining the initial velocities would give the degree of convergence observed. Furthermore, as pointed out by Hass and Miller [44], in their experiments, Chiba and co-workers used commercial 3-PGA, which is contaminated with 2,3-BPGA, without further purification.

Table 2 summarizes the values of K_m and of K_i for 3-PGA and for 2,3-BPGA of native BB-PGAM estimated from appropriate secondary plots, in comparison with the values reported by others. As shown, the values found by us are of the same order of magnitude as those found for MM-PGAM from rabbit muscle [43], and for MM, BB and MB-PGAM isoenzymes from pig heart [29] under the same experimental conditions.

The small amount of PGAM isolated from patient's hemolysates by hydroxyapatite chromatography impeded to realize the full series of initial velocity measurements realized with the native enzyme. Therefore, to compare the kinetic constants of mutated and native PGAM, the apparent K_m values for 3-PGA at 0.5 μM and 10 μM 2,3-BPGA, and the apparent K_m values for 2,3-BPGA at 0.1 mM and 1 mM 3-PGA were determined at pH 7.1. The results, summarized in Table 3, show similar values for the native and the mutated PGAM. Although some differences were observed, they probably do not have physiological significance. We could not determine if the mutated PGAM has a different k_{cat} value than the native enzyme, because the lack of enough patient's blood to fully purify the PGAM. Therefore, it is not possible to say whether, in addition to the decreased stability of the mutated PGAM, the loss of PGAM activity in the erythrocytes of the patient involves a decrease of the k_{cat} of the enzyme.

Finally, the determination of the influence of pH on the initial rates of the native and the mutated BB-PGAM showed that both enzymes have a similar pH optimum. Their initial rates possess a maximum value at pH 6.5, and they fall to one-half at about pH 5.3 and 8.0 (data not presented).

3.4. 2,3-BPGA phosphatase activity

As commented in the Introduction, the PGAM isoenzymes possess some collateral 2,3-BPGA phosphatase activity, which is a consequence of the instability of the phosphoenzyme intermediate in the reaction mechanism [45,46]. This phosphatase activity is much smaller than that of the BPGAM and of the BPGAM/B-PGAM hybrid enzymes and represents a minor proportion of the total 2,3-BPGA phosphatase activity of the erythrocytes [45,47]. To compare the 2,3-BPGA phosphatase activity of the native and the mutated PGAM, the ratio monophosphoglycerate mutase activity (pH 7.2)/2,3-BPGA phosphatase activity (pH 6.8) of the initial fractions of the first peak eluted from the hydroxyapatite columns was determined.

Table 2
Kinetic constants of PGAM isoenzymes

Isoenzyme	Source	3-PGA		2,3-BPGA	
		K_m (mM)	K_i (mM)	K_m (μM)	K_i (μM)
BB-PGAM	Human erythrocyte [a]	0.4 (0.7)	~0.3	1 (0.5)	~100
BB-PGAM	Pig heart [b]	0.1	0.4	0.4	64
MM-PGAM	Pig heart [b]	0.2	0.8	0.5	136
MB-PGAM	Pig heart [b]	0.1	0.5	0.4	61

[a] Mean value of two controls. Numbers inside parentheses are the absolute differences of the two values divided by the mean.
[b] Data from Ref [29].

Table 3
Kinetic constants of native and mutated BB-PGAM

		K_m 3-PGA (mM)		K_m 2,3-BPGA (μM)	
		2,3-BPGA		3-PGA	
		0.5 μM	10 μM	0.1 mM	1 mM
Native (1)	Preparation 1 [a]	0.1	0.3	0.2	1.3
	Preparation 2 [b]	0.1	0.1	0.05	0.4
Native (2)	Preparation 1 [a]	0.2	0.5	0.2	1.2
	Preparation 2 [b]	0.1	0.5	0.1	0.8
Mutated	Preparation 1 [b]	0.3	0.5	<0.1	0.1
	Preparation 2 [b]	0.2	0.3	0.03	0.2

[a] From DEAE-Sephacel chromatography.
[b] From hydroxyapatite chromatography.

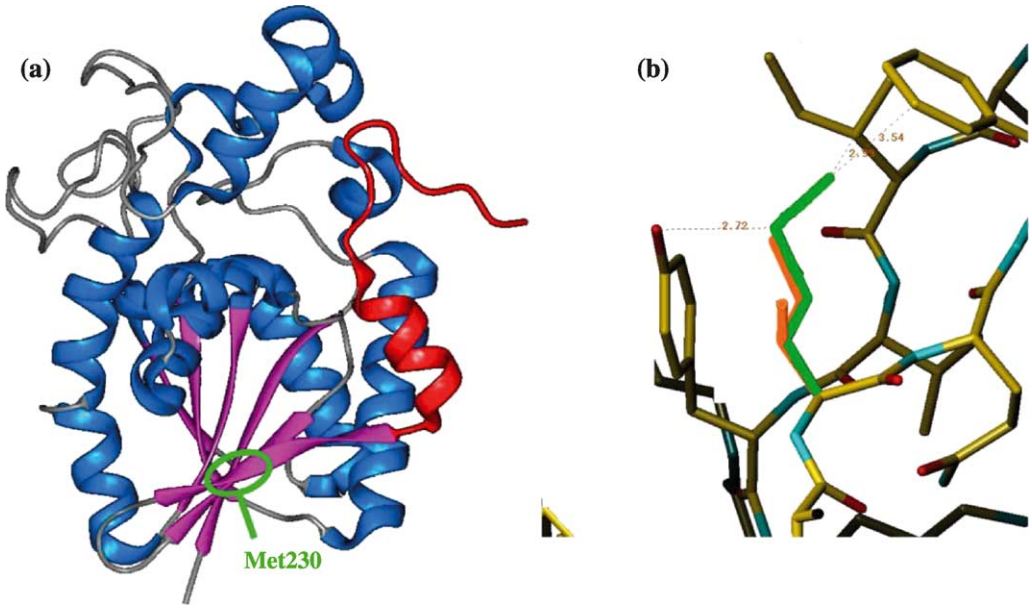

Fig. 6. Panel "a": Ribbon plot of the structure of type B PGAM subunit showing the location of the C-terminal tail (in red) and of Met230 in the last β-strand of the core β-sheet of PGAM subunit. Panel "b": Draw by sticks showing the side-chain of Met230 (in green) and the modelled side-chain of Ile230 (in orange), surrounded by the side-chains of Tyr218 and Phe232.

The value found for the native PGAM (114) was much higher than that of the mutated enzyme (18). This could result from the decrease of the phosphoglycerate mutase activity, but it also might be due to an increase of the 2,3-BPGA phosphatase activity produced by the mutation. To differentiate these two possibilities would require amounts of purified enzyme much larger that those that we could obtain from patient's blood.

On the other hand, the 2,3-BPGA phosphatase activity of the native and the mutated PGAM at pH 6.8 and at pH 7.6 was compared. The activity ratio pH 6.8/pH 7.6 determined for the native PGAM (1.6) was similar to that found for the mutated enzyme (1.2).

3.5. PGAM modelling

As shown in Fig. 6, the modelling of human type B PGAM subunit based on the crystal structure of PGAM from *E. coli* [26] has confirmed the results of the previous modelling based on the structure of PGAM from *S. cerevisiae* [11]. Met 230 is located in the last β-strand of the core β-sheet of PGAM subunit that precedes the C-terminal region. This region is not modelled in the structure of the inactive dephosphorylated PGAM from *S. cerevisiae* [34–38] because of disorder. But, with the exception of the final two residues, it is ordered in the active phosphorylated form of PGAM from *E. coli* [26,39]. In the active form, the C-terminal region is constituted by a α-helix (helix 10) and a short β-hairpin motif. This motif extends away from the helix 10 across the active site opening, forming a number of hydrogen bonds with residues of the rim and substrate-binding region. In the native PGAM, the side chain of Met230 forms a π-interaction [48], packing between Tyr218 and Phe 232, which stabilizes the β-strand. The substitution of Met by Ile in the mutated PGAM breaks the π-interaction, with an energetic cost of about 0.3–0.5 kcal/mol [48], and the last strand packing becomes unsteady. This could affect indirectly the enzymatic activities and decrease PGAM stability.

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