Biochimica et Biophysica Acta 1830 (2013) 2608–2618

Contents lists available at SciVerse ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbagen

Review

Multiple roles of glucose-6-phosphatases in pathophysiology State of the art and future trends

Paola Marcolongo [a], Rosella Fulceri [a], Alessandra Gamberucci [a], Ibolya Czegle [b], Gabor Banhegyi [c], Angelo Benedetti [a,*]

[a] Department of Pathophysiology, Experimental Medicine and Public Health, University of Siena, Siena, Italy
[b] 3rd Department of Internal Medicine, Semmelweis University, Budapest, Hungary
[c] Department of Medical Chemistry, Molecular Biology and Pathobiochemistry, Semmelweis University, Budapest, Hungary

ARTICLE INFO

Article history:
Received 8 October 2012
Received in revised form 11 December 2012
Accepted 13 December 2012
Available online 21 December 2012

Keywords:
Glucose-6-phosphatase
Endoplasmic reticulum
Glycemia
von Gierke disease
Diabetes
Congenital neutropenia

ABSTRACT

Background: The endoplasmic reticulum enzyme glucose-6-phosphatase catalyzes the hydrolysis of glucose-6-phosphate to glucose and inorganic phosphate. The enzyme is a part of a multicomponent system that includes several integral membrane proteins; the catalytic subunit (G6PC) and transporters for glucose-6-phosphate, inorganic phosphate and glucose. The *G6PC* gene family presently includes three members, termed as *G6PC*, *G6PC2*, and *G6PC3*. Although the three isoforms show a moderate amino acid sequence homology, their membrane topology and catalytic site are very similar. The isoforms are expressed differently in various tissues. Mutations in all three genes have been reported to be associated with human diseases.

Scope of review: The present review outlines the biochemical features of the *G6PC* gene family products, the regulation of their expression, their role in the human pathology and the possibilities for pharmacological interventions.

Major conclusions: G6PCs emerge as integrators of extra- and intracellular glucose homeostasis. Beside the well known key role in blood glucose homeostasis, the members of the G6PC family seem to play a role as sensors of intracellular glucose and of intraluminal glucose/glucose-6-phosphate in the endoplasmic reticulum.

General significance: Since mutations in the three *G6PC* genes can be linked to human pathophysiological conditions, the better understanding of their functioning in connection with genetic alterations, altered expression and tissue distribution has an eminent importance.

1. Introduction

The "classic" glucose-6-phosphatase enzyme (G6PC, G6Pase, EC 3.1.3.9), highly expressed in glucogenic organs, i.e. liver and kidney, has been extensively investigated since the fifties [see 1 for a review].

More recently, isoforms of G6PC have been identified and a variety of studies investigated their functions [see 2 for a review]. In particular at least two other isoforms – coded by different genes – have been described; their properties are reported in Table 1. The aim of the present review is to summarize the biochemical features of the *G6PC* gene family products, the regulation of their expression, the role of mutations in the corresponding genes in the human pathology and the future possibilities for pharmacological interventions.

2. The "classic" G6Pase, G6PC

The enzyme is known for a long time to be localized in the endoplasmic reticulum (ER) membrane [3], and its catalytic site faces the lumen of the ER [4]. Its positioning, therefore, requires the permeation of the cytosolic substrate glucose-6-phosphate (G6P) [1,5] as well as of the hydrolysis products inorganic phosphate (Pi) and glucose [1,5] across the ER membrane (Fig. 1). In agreement with this assumption, a G6P transporter (G6PT, SLC37A4) has been identified, cloned [6] and immunolocalized [7]. G6P has been shown to be counter-transported

Abbreviations: G6PC, glucose-6-phosphatase, G6Pase; G6PC2, glucose-6-phosphatase isoform 2; G6PC3, glucose-6-phosphatase isoform 3; ER, endoplasmic reticulum; G6P, glucose-6-phosphate; Pi, inorganic phosphate; G6PT, glucose-6-phosphate transporter; GK, glucokinase; PEPCK, phospho*enol*pyruvate carboxykinase; SI, small intestine; SRC-2, steroid receptor coactivator 2; RORα, retinoid-related orphan receptor α; GSD1, glycogen storage disease type 1; GSD1a, inherited deficiency of G6PC; GSD1b, inherited deficiency of G6PT; NOD, non-obese diabetic mice; SNP, single-nucleotide polymorphisms; FGL, fasting glucose level

* Corresponding author at: Viale A. Moro 2, 53100-Siena, Italy. Tel.: +39 0577 234021; fax: +39 0577 234009.
E-mail address: benedetti@unisi.it (A. Benedetti).

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http://dx.doi.org/10.1016/j.bbagen.2012.12.013

Table 1
Properties of the glucose-6-phosphatase isoforms.

Isoform	G6PC	G6PC2	G6PC3
Synonymous	G6PC1, G6Pase α	Islet specific G6PC related protein (IGRP).	G6Pase β, ubiquitously expressed G6PC related protein (UGRP).
Human gene chromosomal location	17q21 [153]	2q31 [108]	17q21 [133]
Mw of the product (kDa)	35 kDa [152]	40.7 [2]	38.7 [2]
Amino acid sequence homology % of G6PC	100	50 [2]	36 [2]
Membrane topology	Nine ER transmembrane domains [4,154]. Intralumenal residues predicted to play a role in catalysis: Arg83, His_{119} and His_{176} [2].	Nine ER transmembrane domains [155]. Catalytically important residues akin to G6PC [107].	Nine ER transmembrane domains [57,156]. Catalytically important residues akin to G6PC [57,156].
Tissue distribution	Liver, kidney, pancreatic ß-cells, intestinal mucosa [1].	Pancreatic ß-cells [2,108].	Ubiquitous [57,133].
G6Pase activity	Well known activity [see 1].	No activity of the recombinant protein [107]. Activity present in the recombinant protein [2,109]; Km and Vmax lower than G6PC [109].	No activity of the recombinant protein [133]. Activity present in the recombinant protein [57,136]; Km higher, but Vmax lower than G6PC [57]. Skeletal muscle activity of endogenous G6PC3 approx. 40-times lower than liver G6PC [136].
Involvement in pathophysiology	Inherited deficiency: glycogen storage disease type 1a (GSD1a) [81,82]. Overexpression in type 2 diabetes [2,95,96].	Autoantigen in type 1 diabetes? [111–117]. Regulation of fasting glucose levels [118–125].	Inherited deficiency: congenital neutropenia and developmental alterations [144–150].

with Pi in a model system (liposomes including the reconstituted G6PT protein) [8], but very recent evidence indicates that G6PT acts as a facilitative uniporter in native liver ER derived vesicles (microsomes) [9]. According to the counter transport hypothesis [8], Pi transport would be dependent on that of G6P, but direct evidences for a G6P independent Pi permeability of liver microsomes have been forwarded [9,10]. Moreover, anion channels permeable to Pi are present in the sarcoplasmic reticulum membrane [11]. ER glucose permeability has been characterized in liver microsomes [12–14] and in cell models [15] but the putative transporter(s) is still elusive. Recently, the glucose transporter, GLUT10, has been localized to ER in model cells [16], and in HepG2 cells (*Marcolongo el al preliminary results*), its role however is still to be defined. Alternatively, plasma membrane glucose transporters may also function as ER glucose transporter during their posttranslational transit [17].

The properties of the catalytic subunit of the G6Pase system, referred as G6PC, are outlined in Table 1.

2.1. Liver and kidney G6PC

The main physiological function of liver G6PC is to regulate whole body glucose homeostasis, in particular to maintain the blood glucose level constant [18]. This can be done since G6PC catalyzes the common terminal reaction of gluconeogenesis and glycogenolysis (Fig. 1). The high expression of both the G6PC and G6PT proteins in the liver ER, together with their uneven distribution in the ER membranes [19] can guarantee an efflux of glucose from the liver adequately high to affect the glycemia under fasting conditions. Kidney G6PC can also contribute to whole-body glucose turnover up to 25% in the deep fasting status [e.g. 20,21] and diabetes [e.g. 22], conditions under which kidney works as a major gluconeogenetic site. A role for kidney G6PC is also demonstrated by the fact that in the anhepatic phase of liver transplantation the renal glucose production can compensate the liver glucose production [23,24]. These results have been recently confirmed and further characterized in liver specific *G6pc*$^{-/-}$ mice [25]. These mice, different from the global *G6pc*$^{-/-}$ mice [26], do not present with a marked hypoglycemia, can survive in the absence of dietary glucose and exhibit a marked glucagon-induced increase of renal gluconeogenesis during fasting status.

2.2. G6PC in non-glucogenic tissues

Many old studies addressed the presence of G6Pase activity in tissues other than liver and kidney. In many instances, G6Pase activity was measured by properly taking into account G6P hydrolysis due to unspecific phosphatases. It has been done by inactivating the unspecific phosphatases with preincubation at pH 5.0, by evaluating the hydrolysis of poor substrates of G6Pase (e.g. glucose-1-phosphate or β-glycerophosphate), by using highly purified microsomal fractions and by electron microscopy localization of the enzyme activity products within the ER cisternae. On the basis of these observations, as well as of more recent data on the expression of G6PC mRNA and protein, G6PC appears to be also present – although at a low extent – in pancreatic ß-cells and intestinal mucosa [1].

2.2.1. Pancreatic ß-cells

It has been observed for a long time that rodent pancreatic islets possess G6Pase activity [27–29]. Rodent ß-cells also express the G6PC/G6PT mRNA [30]. Both the enzyme activity and the G6PC protein appear to become expressed – together with the glucose-induced insulin secretion – in the INS1 rat insulinoma cell line as compared to the parent, glucose-insensitive, RINm5F cells [31].

It has been proposed that islet G6Pase activity – similar to the liver one [32,33] – can stimulate the Mg-ATP dependent ER calcium accumulation and regulate calcium signaling-activated insulin secretion [29,34]. Glucose feeding enlarges the agonist-sensitive ER calcium pool in ß-cells [e.g. 35,36] and INS1 cells [37], and the uptake of calcium by the ER is also modulated by the glucose concentration of the medium of mouse pancreatic ß-cells suspensions [38]. In theory, these effects might be due to either the increase in ATP:ADP ratio or in the cytosolic levels of G6P [29]. Both parameters oscillate upon glucose challenging [39]. In any event, the possible relationships between the ß-cells G6Pase activity and calcium homeostasis remain largely unexplored.

It has been also hypothesized that G6Pase activity in combination with glucokinase (GK), creates a futile substrate cycle in which ATP is utilized, thereby reducing the ATP:ADP ratio and hence glucose-stimulated insulin secretion by ß-cell [40–43]. Alternatively – or in addition – G6Pase can function antagonistically to GK by acting as a sink for G6P thus reducing