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Review

The function and the role of the mitochondrial glycerol-3-phosphate dehydrogenase in mammalian tissues

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ABSTRACT

Mitochondrial glycerol-3-phosphate dehydrogenase (mGPDH) is not included in the traditional textbook schemes of the respiratory chain, reflecting the fact that it is a non-standard, tissue-specific component of mammalian mitochondria. But despite its very simple structure, mGPDH is a very important enzyme of intermediary metabolism and as a component of glycerophosphate shuttle it functions at the crossroads of glycolysis, oxidative phosphorylation and fatty acid metabolism. In this review we summarize the present knowledge on the structure and regulation of mGPDH and discuss its metabolic functions, reactive oxygen species production and tissue and organ specific roles in mammalian mitochondria at physiological and pathological conditions.

1. Introduction

It is hard to believe that the research on the mitochondrial glycerol-3-phosphate dehydrogenase (mGPDH) started already 75 years ago [1] and yet there are still many questions to be answered, both concerning the structure/function relationship and regulation of this enzyme. This becomes even more striking when we consider its simple structure. With only one 74 kDa subunit, mGPDH is the simplest component of the mammalian respiratory chain.

mGPDH is an integral component of the mammalian respiratory chain and glycerophosphate (GP)-shuttle connects mitochondrial and cytosolic processes and plays an important role in cell bioenergetics, both under physiological and pathological conditions. GP-shuttle function depends on a highly variable expression of mGPDH in various mammalian tissues. It can be regulated by two mechanisms — by the content of mGPDH protein, because cytosolic cGPDH is not rate limiting for GP-shuttle function and by allosteric regulation of mGPDH activity by several metabolites or ions. However, it is still not completely understood why mGPDH expression is so highly suppressed in most mammalian tissues and what is the real reason for extremely high mGPDH activity in others.

Three possible metabolic roles for high mGPDH/GP-shuttle function have so far been suggested: (i) reoxidation of cytosolic NADH in glycolytic cells; (ii) bypassing complex I during cytosolic NADH oxidation with possible implications in thermogenesis and/or metabolic efficiency; and (iii) regulation of cytosolic glycerol-3-phosphate (G3P) as metabolite connecting glycolysis, lipogenesis and oxidative phosphorylation (OXPHOS).

Another important feature we will focus on is the reactive oxygen species (ROS) generation by mGPDH that is not sufficiently protected against electron leak and could also facilitate ROS production at other sites of the respiratory chain. This may also be one of the reasons why in most tissues its expression is suppressed.

Finally we will discuss tissue or organ-specific aspects of mGPDH metabolic involvement at physiological and pathological states which enables us to define the specific roles of this enzyme in mammalian organism.

2. Simple, yet unclear structure and reaction mechanism of mGPDH

Mitochondrial glycerol-3-phosphate dehydrogenase is a flavin-linked respiratory chain dehydrogenase that oxidizes glycerol-3-phosphate (G3P) to dihydroxyacetone phosphate (DAP) (redox potential −190 mV) with concurrent reduction of flavin adenine dinucleotide (FAD) to $FADH_2$ and transfers electrons to coenzyme Q (CoQ).

Mammalian mGPDH is encoded by a single *GPD2* gene located on chromosome 2 in human and chromosome 3 in rat. It is a conserved

Abbreviations: mGPDH, mitochondrial FAD-dependent glycerol-3-phosphate dehydrogenase; cGPDH, cytosolic NADH-dependent glycerol-3-phosphate dehydrogenase; CoQ, coenzyme Q; GP-shuttle, glycerophosphate shuttle; G3P, glycerol-3-phosphate; DAP, dihydroxyacetone phosphate; BAT, brown adipose tissue; FFA, free fatty acid; RET, reverse electron transport; OXPHOS, oxidative phosphorylation; ROS, reactive oxygen species; SDH, succinate dehydrogenase; T_3, 3,5,3′-tri-iodo-L-thyronine; $\Delta\Psi$, mitochondrial membrane potential

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and only slowly evolving gene with mammalian mGPDH showing a high degree of homology with yeast, insect and bacterial flavin-linked G3P dehydrogenases. On the other hand, there is no homology with the cytoplasmic NAD-linked glycerol-3-phosphate dehydrogenase (cGPDH) encoded by *GPD1* gene, the soluble dehydrogenase that reduces DAP to G3P while oxidizing cytosolic NADH (Fig. 1).

Rat mGPDH is synthesized as 79 kDa precursor that is processed to ~74 kDa mature protein upon removal of the N-terminal (~5 kDa) presequence, after the import into mitochondria [2–4]. Following the early attempts to isolate mGPDH [5,6], mammalian enzyme was isolated from several tissues of rat, pig or rabbit [2,7–9] yielding in best cases very pure mGPDH protein with varying content of noncovalently bound FAD, non-heme Fe and acid-labile sulfide that ranged from substoichiometric amounts to approximately one molecule of cofactor and acid-labile sulfide per mol of enzyme [2,7,10]. This is a uniquely low content of prosthetic groups (cofactors) among mitochondrial electron transport enzymes that mirrors the simple structure of mGPDH comparable with rubredoxin oxidase of the strict anaerobe *Desulfovibrio gigas* [11,12]. Despite the early studies indicating the presence of non-heme iron, there is no FeS center present in the crystal of bacterial enzyme and neither there is consensus CxxCxxC motif for 4Fe4S center coordination in the sequence of mammalian protein. Therefore FAD is most likely the only prosthetic group present in mGPDH.

As the crystal structure of mammalian mGPDH is not yet available, the membrane topology can only be deduced from the hydropathy plot of amino acid sequence, biochemical studies and 3-D structure of its bacterial homologue GlpD [13] or closely related bacterial G3P oxidase (GlpO) [14].

Crystal structures of bacterial GlpD from *E. coli* [13] and GlpO from *Streptococcus* sp. [14] suggest anchoring to the membrane without transmembrane helices and the presence of dimeric form of enzyme that interacts through the hydrophobic regions, supporting the view that mGPDH is strongly associated with the inner membrane but rather as a peripheral, than an integral protein. Although this fold is rather unusual it is shared with another dehydrogenase communicating with the CoQ pool — electron transfer flavoprotein (ETF):Q dehydrogenase [15,16] and several other enzymes utilizing hydrophobic ligands. All these proteins share structural motifs that run parallel to the membrane surface and form apolar plateaus that are hypothesized to bury into one leaflet of the membrane only [17]. This view is also supported by the fact, that yeast mGPDH is extracted from the membrane by carbonate but not by high salt treatment implying that the enzyme is peripheral and holds in the membrane by hydrophobic interactions [18].

Nevertheless detailed structural information for the mammalian enzyme is still missing. Given the relatively good sequence conservation between mammalian and bacterial enzyme (30% identity and 48% similarity), it has most likely monotypic structure as well. The C terminal Ca^{2+} binding domain is not present in the bacterial enzyme and its structure is unclear. It should be noted that early studies of MacDonald and Brown [19], predicted three transmembrane segments in the N terminal third of the protein with the long C-terminal region exposed to the intermembrane space. However, this fold which was based on von Heine's algorithm [20] is rather unlikely, as parts of the predicted transmembrane helices comprise FAD binding site which is very well conserved from the bacterial enzyme and presumably has the same fold not protruding the membrane. Some of the hydrophobic parts,

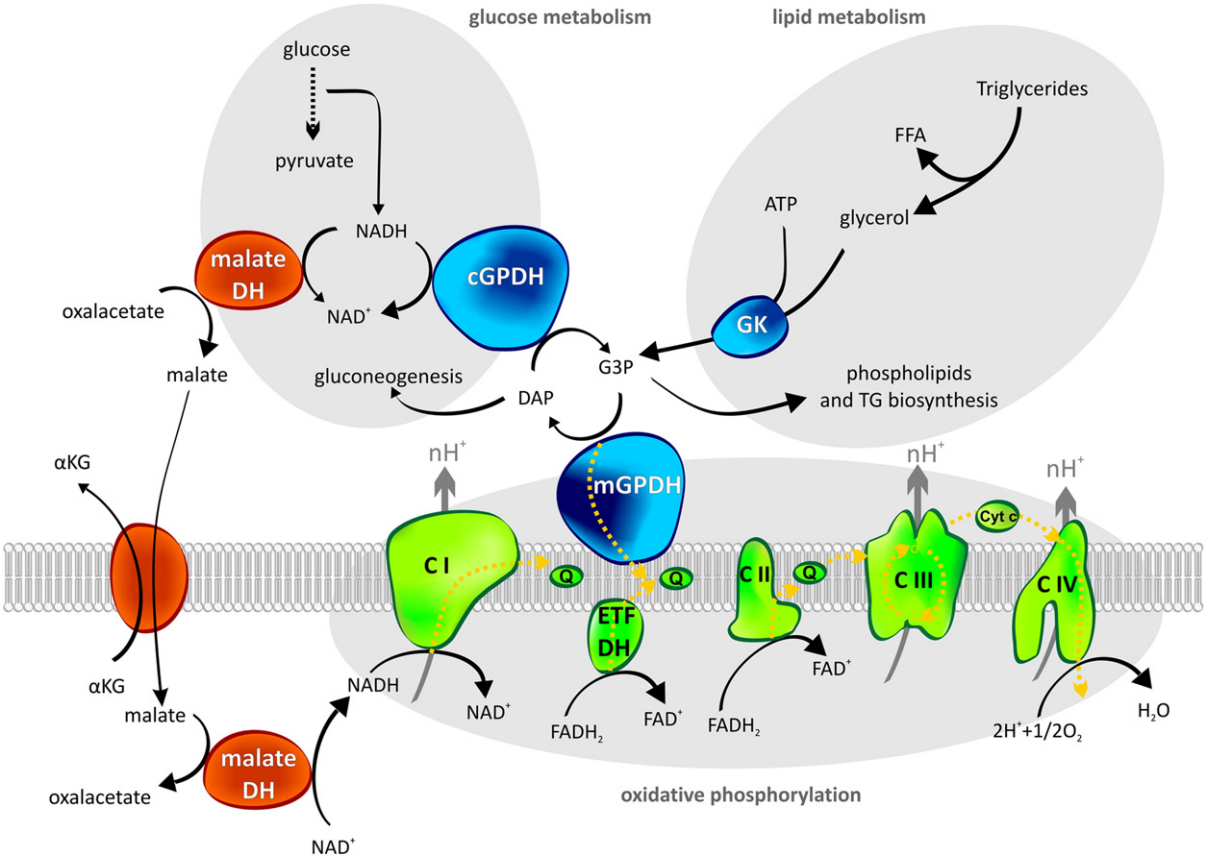

Fig. 1. mGPDH and its place in intermediary metabolism. Enzymes constituting GP-shuttle are depicted in blue as well as glycerol kinase (GK) which phosphorylates glycerol coming from lipolysis. Part of the malate/aspartate-shuttle as an alternative pathway of reducing equivalents transport is depicted in orange. Complexes of the mitochondrial respiratory chain are in green with yellow arrows indicating flow of electrons through the OXPHOS. Q — Coenzyme Q, CI — complex I, CII — complex II, CIII — complex III, CIV — complex IV, ETF DH — ETF:Q dehydrogenase, FFA — free fatty acids, G3P — glycerol-3-phosphate, DAP — dihydroxyacetone phosphate.