Mammalian P5CR and P5CDH: Protein Structure and Disease Association

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Abstract

The interconversions of L-proline (Pro) and L-glutamate (Glu) in mammalian cells involve an obligatory intermediate, Δ^1 -pyrroline-5-carboxylate (P5C/PYC), and four uni-directional enzymes, proline oxidase/dehydrogenase (POX/PRODH), P5C dehydrogenase (P5CDH), P5C synthase (P5CS/PYCS), and P5C reductase (P5CR/PYCR). The catabolism of Pro by two dehydrogenation reactions catalyzed by POX and P5CDH is an important source of oxidizing signaling, whereas the anabolism of Pro through two reduction reactions catalyzed by P5CS and P5CR maintains redox homeostasis to promote cell growth. In addition, Pro is one of the “conditionally essential” amino acids in the neonatal intestine in both humans and animals. The homeostatic balance of Pro, Glu, and Arginine is critical for the growth, redox balance, immunomodulation, and development of mammals. Recent discoveries strongly suggest that Pro metabolic enzymes are tightly regulated by spatial-temporal gene expression, tissue and cell-specificity, substrate and/or inhibitor abundance, and subcellular compartmentalization. In terms of disease association, mutations in human P5CR1 gene have been identified in patients with autosomal recessive Cutis laxa type IIB and type IIB/De Barsy syndrome, whereas mutations in P5CDH gene cause type II hyperprolinemia. Importantly, x-ray crystallographic studies have revealed the protein structures of human P5CR 1 and mammalian P5CDH. These new discoveries in structure function relationship may provide crucial guidelines in the treatment of the corresponding disorders.

Keywords: Cutis laxa Type IIB and Type IIB/De Barsy syndrome; DJ-1; ORAOV1; P5CR/PYCR; P5CDH; Subcellular localization

Abbreviations

P5C/PYC: Δ^1 -pyrroline-5-carboxylate; P5CDH: P5C Dehydrogenase; P5CR: P5C Reductase; P5CS: P5C Synthase; OAT: Ornithine Aminotransferase; GSA: Glutamic- γ -Semialdehyde

Introduction

In mammals, L-proline (Pro) and L-glutamate (Glu) are interconverted by four highly regulated, uni-directional enzymes, namely, Pro oxidase/dehydrogenase (POX/PRODH), Δ^1 -pyrroline-5-carboxylate (P5C/PYC), P5C Dehydrogenase (P5CDH), P5C Synthase (P5CS/PYCS), and P5C Reductase (P5CR/PYCR), with P5C as the obligatory intermediate [1-4] (Table 1).

P5C is in tautomeric equilibrium with Glutamic- γ -Semialdehyde (GSA), which is reduced to Pro by the cytosolic and mitochondrial NAD(P)H-dependent P5CR isozymes. In addition, P5C/GSA is a substrate for two other enzymes, mitochondrial P5CDH, which converts P5C/GSA to Glu [5], and mitochondrial Ornithine (Orn) Aminotransferase (OAT), which catalyzes the interconversion of P5C and Orn [6]. Orn enters the urea cycle mainly for ammonia detoxification and Arginine (Arg) biosynthesis (Figure 1). It has been shown that Pro and Arg are two of the “conditionally essential” amino acids in the neonatal intestine of mammals. The interconversions of Pro, Orn, Glu, and Arg are critical for the growth and immunomodulation of mammals [1,4,7]. At the cellular level, the catabolism/degradation of Pro by POX and then P5CDH is an important source of redox signaling, whereas the anabolism/biosynthesis of Pro through P5CS and then P5CR maintains redox homeostasis to promote cell growth. In this review, we summarize the recent discoveries on human P5CR isozymes and P5CDH and their associated diseases/disorders.

Human P5CR: Three isozymes encoded by three different genes

Three isozymes of human P5CR/PYCR (EC1.5.1.2), have been identified, cloned, and characterized [1,2,8]. Human P5CR1, P5CR2, and P5CR3 are encoded by three different genes, localized at three different chromosomal locations (Table 1). These three isozymes catalyze ATP and NAD(P)H-dependent reduction of P5C to Pro, which is important for the transfer of oxidizing potential across the cell [9,10]. The human P5CR1/PYCR1 structural gene [also known as Proliferation-Inducing protein 45 (PIG45)] is localized on chromosome 17q25.3, which encodes two protein isoforms, a 319-amino acid residue (aa) and a 316-aa polypeptide, respectively. The human P5CR2/PYCR2 structural gene is localized on chromosome 1q42.12, which encodes two protein isoforms, a 320-aa and a 246-aa polypeptide, respectively. Recently, De Ingeniis and colleagues [8] confirmed that there is a new isozyme of P5CR, P5CR3, in melanoma cells. The human P5CR3/PYCR3 structural gene is localized on chromosome 8q24.3, which encodes two isoforms, a 286-aa and a 266-aa polypeptide, respectively. In addition, it has

Table 1: Human P5CR Isozymes and P5CDH: from genes to protein isozymes to associated disorders.

Enzyme	Gene Name	Gene ID	Map Location	OMIM#	Isozyme	# Amino Acids
P5CR1	PYCR1/P5CR1	5831	17q25.3	179035	P5CR1.1	319
				612940	P5CR1.2	316
				614438		
P5CR2	PYCR2/P5CR2	29920	1q42.12	N/A	P5CR2.1	320
					P5CR2.2	246
P5CRL/P5CR3	PYCRL/P5CR3	65263	8q24.3	N/A	P5CRL.1	286
					P5CRL.2	266
P5CDH	ALDH4A1/P5CDH	8659	1p36	606811	P5CDH	563

Figure 1: The interconversions of proline, glutamate, and ornithine. This tri-amino-acid-cycle also links with the urea cycle and the pentose phosphate pathway.

been demonstrated that P5CR1 and P5CR2 are localized in the mitochondria and are primarily involved in conversion of Glu to Pro, whereas P5CRL is localized in the cytosol and is exclusively linked to the conversion of Orn to Pro, and is not feedback inhibited by proline. Previously, Merrill and colleagues [11] showed that human P5CR in erythrocytes not only catalyzes the obligatory step in Pro biosynthesis, but also plays a physiological role in the generation of NADP⁺. The normal abundance of P5CR in the cell is maintained relatively low due to its high turnover [9]. A recent study by Krishnan and colleagues [12] showed over expression of P5CR1 resulted in 2-fold higher proline content, significantly lowered free radical levels, and increased cell survival. Another studies showed that increased P5CR1 activity was measurable in pulmonary and colorectal tumors [13,14]. In contrast, mammalian P5CR2 and P5CRL are relatively new and not well studied.

With regard to their interactomes, interestingly, PYCR1 has been identified as an interacting protein of two important regulatory proteins, DJ-1 [15] and Oral Cancer Over expressed 1 (ORAOV-1) [16]. DJ-1, encoded by the DJ-1/PARK7 gene, plays various functions involved in transcriptional regulation, anti-oxidative activity, and regulation of mitochondrial complex I. It has been shown that DJ-1 and PYCR1 interacts and colocalizes in mitochondria. DJ-1 increases the enzymatic activity of PYCR1 *in vitro* [15]. ORAOV1, encoded by ORAOV1 gene, is frequently amplified in esophageal squamous cell cancer, and has been shown

to regulate the cell cycle, apoptosis and angiogenesis. Cancer cells overexpressing ORAOV1 exhibited significantly increased tumorigenicity and larger tumors with poor differentiation. It has been shown that ORAOV1 also increases the enzymatic activity of PYCR1 and the production of Pro when it binds with P5CR1 [16]. However, whether P5CR1, DJ-1, and ORAOV1 interact with each other and function in the same complex in certain cell types is not known.

Human P5CR1 Protein structure and associated diseases

The crystal structure of human P5CR1 have been reported recently [13,14]. The 2.8 Angstroms (Å) resolution structure of the P5CR1 apo enzyme and its 3.1 Å resolution ternary complex with NAD(P)H and substrate-analog demonstrated that human P5CR1 possesses a decameric architecture with five homodimer subunits. It has been hypothesized that human P5CR1 possesses ten catalytic sites arranged around a peripheral circular groove. In terms of disease association, homozygous or compound heterozygous mutations in human P5CR1 have been identified in autosomal recessive Cutis laxa, Type IIB (ARCL2B or Cutis laxa with progeroid features; OMIM #612940; Table 1) [17-19]. The clinical phenotype of ARCL2B includes cutis laxa, abnormal growth and development, and associated skeletal abnormalities [18]. In addition, P5CR1 mutations have been linked to autosomal recessive cutis laxa type III (ARCL3, OMIM #614438), also known

as De Barsy syndrome. ARCL3 is a rare autosomal recessive disorder, characterized by an aged appearance with distinctive facial features, sparse hair, ophthalmologic abnormalities, intrauterine growth retardation, and cutis laxa.

Human P5CDH: Protein structure and associated disease

Mammalian P5CDH (EC 1.5.1.12) is a mitochondrial matrix NAD⁺-dependent dehydrogenase which converts P5C/GSA to Glu, and thus is a high Km/low affinity Aldehyde Dehydrogenase (ALDH) with GSA as a primary substrate. Mammalian P5CDH also exhibits activity with other aldehydes, and is dubbed as ALDH4A1 (ALDH, family 4, subfamily A, member 1) [1,2] (Table 1). Human P5CDH/ ALDH4A1 structural gene is localized on chromosome 1p36 and encodes a 563-aa polypeptide [1,5]. Importantly, it has been demonstrated in plants that Lack of P5CDH activity led to higher ROS production in the presence of Pro excess. Therefore, oxidation of P5C to Glu by P5CDH is critical to prevent P5C-Pro intensive cycling and avoid ROS production from electron run-off [20-22]. In additional, it has been shown that *Drosophila* deficient in P5CDH showed hyperprolinemia, swollen mitochondria, and early embryonic lethality [23]. Taken together, these observations suggest that P5CDH plays a protective role against ROS generation, mitochondria and cell damage, and apoptosis.

To understand the functions of P5CDH at the molecular level, how substrates and inhibitors interact with the enzyme, and how the substituted residues encoded by the mutant alleles of P5CDH can affect the enzymatic activity, the crystal structures of human and mouse P5CDH were determined recently [24,25]. Both wildtype P5CDH and mutant P5CDH proteins carrying S352A (2.4 Å) and S352L (2.85 Å) substitutions were resolved. In addition, 2.5-Å resolution Structures of the mouse P5CDH complexed with sulfate ion (1.3 Å resolution), glutamate (1.5 Å), and NAD⁺ (1.5 Å) were determined in order to obtain high-resolution views of the active site. Together, the structures showed that single amino acid substitutions cause structural alterations and enzyme inactivation. Interestingly, the structure-activity relationship demonstrated that the semialdehyde carbon chain length and the position of the aldehyde group in relation to the cysteine nucleophile and oxyanion hole of mouse P5CDH are critical. Efficient 4- and 5-carbon substrates share the common feature of being long enough to span the distance between the anchor loop at the bottom of the active site and the oxyanion hole at the top of the active site. The inactive 2- and 3-carbon semialdehydes bind the anchor loop but are too short to reach the oxyanion hole [25]. The *K_i* values are 0.27 mM for glyoxylate, 58 mM for succinate, 30 mM for glutarate, and 12 mM for L-glutamate. Interestingly, malonate is not an inhibitor [25]. With regard to its disease association, deficiency of P5CDH causes type II Hyperprolinemia (HPH), an autosomal recessive disorder characterized by accumulation of P5C and Pro [6,26,27]. Although HPH has been considered as a benign disorder, further research indicated that HPH may cause clinical manifestations, such as childhood febrile seizures.

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