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Understanding carbamoyl phosphate synthetase (CPS1) deficiency by using the recombinantly purified human enzyme: Effects of CPS1 mutations that concentrate in a central domain of unknown function

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ABSTRACT

Carbamoyl phosphate synthetase 1 deficiency (CPS1D) is an inborn error of the urea cycle that is due to mutations in the *CPS1* gene. In the first large repertory of mutations found in CPS1D, a small CPS1 domain of unknown function (called the UFSD) was found to host missense changes with high frequency, despite the fact that this domain does not host substrate-binding or catalytic machinery. We investigate here by *in vitro* expression studies using baculovirus/insect cells the reasons for the prominence of the UFSD in CPS1D, as well as the disease-causing roles and pathogenic mechanisms of the mutations affecting this domain. All but three of the 18 missense changes found thus far mapping in this domain in CPS1D patients drastically decreased the yield of pure CPS1, mainly because of decreased enzyme solubility, strongly suggesting misfolding as a major determinant of the mutations negative effects. In addition, the majority of the mutations also decreased from modestly to very drastically the specific activity of the fraction of the enzyme that remained soluble and that could be purified, apparently because they decreased V_{max}. Substantial although not dramatic increases in K_m values for the substrates or for N-acetyl-L-glutamate were observed for only five mutations. Similarly, important thermal stability decreases were observed for three mutations. The results indicate a disease-causing role for all the mutations, due in most cases to the combined effects of the low enzyme level and the decreased activity. Our data strongly support the value of the present expression system for ascertaining the disease-causing potential of CPS1 mutations, provided that the CPS1 yield is monitored. The observed effects of the mutations have been rationalized on the basis of an existing structural model of CPS1. This model shows that the UFSD, which is in the middle of the 1462-residue multidomain CPS1 protein, plays a key integrating role for creating the CPS1 multidomain architecture leading us to propose here a denomination of "Integrating Domain" for this CPS1 region. The majority of these 18 mutations distort the interaction of this domain with other CPS1 domains, in many cases by causing improper folding of structural elements of the Integrating Domain that play key roles in these interactions.

1. Introduction

Primary CPS1 deficiency (CPS1D; MIM #237300), a recessively inherited urea cycle disease leading to frequently fatal hyperammonemia [1,2], is due to mutations in the *CPS1* gene. This gene, located in 2q35 [3] and being composed of 38 exons and 37 introns [4–6], with 4,500 coding nucleotides, encodes a 1500-residue proenzyme [7] that is synthesized in hepatocytes and enterocytes [8,9], and which, upon internalization to the mitochondrial matrix, yields after cleavage of its N-terminal 38 amino acids, the mature 1,462-amino acid multidomain (Fig. 1A) CPS1 protein [10–12] [E.C. 6.3.4.16].

CPS1 catalyzes the first step of the urea cycle ($2ATP + NH_3 + HCO_3^- \rightarrow 2ADP + HPO_4^{2-} + NH_2CO_2PO_3^{2-}$) [13], converting ammonia to carbamoyl phosphate (CP), a compound that is utilized by ornithine transcarbamylase (OTC) to make citrulline in the second reaction of the urea cycle. The three-step CPS1 reaction (Fig. 1B) includes two analogous ATP-dependent phosphorylations, of bicarbonate and carbamate, and an intervening step of carbamate synthesis from carboxyphosphate and ammonia [13]. To be active, CPS1 requires the presence of an essential allosteric activator, N-acetyl-L-glutamate (NAG) [10,14,15], made by NAG synthase from glutamate and acetyl-coenzyme A [16]. The rate of NAG synthesis heavily depends on glutamate concentration [17], and therefore NAG represents a switch for CPS1 activity, which is turned

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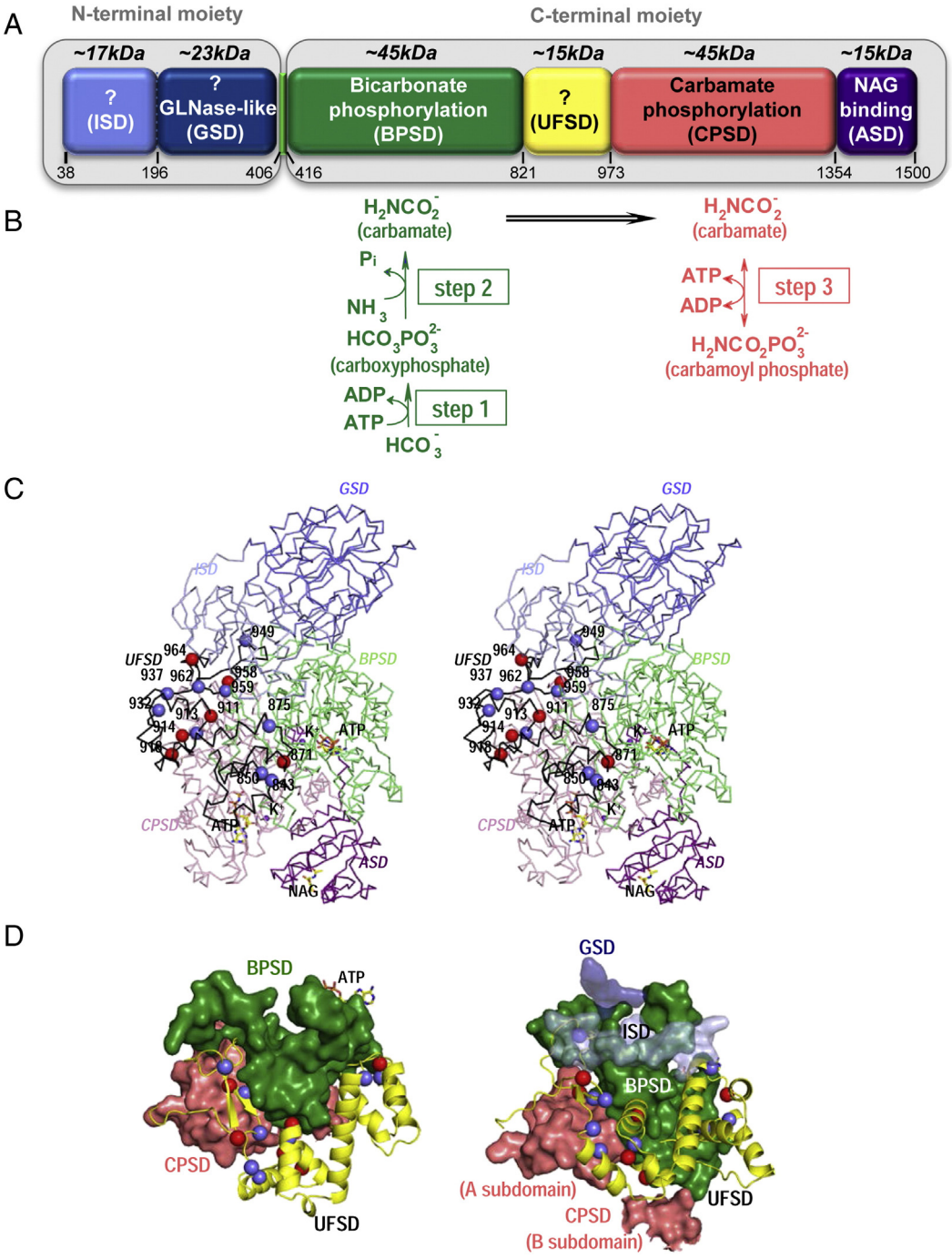

Fig. 1. Domain composition, function and architecture of CPS1. (A) Schematic linear representation of the CPS1 polypeptide, marking its different domains. The grey-shaded background bars schematize the 40-kDa N-terminal and the 120-kDa C-terminal moieties of the enzyme that correspond to the small and large subunits of *E. coli* CPS, respectively. The colored bars represent the different CPS1 domains as defined from sequence alignment with *E. coli* CPS. Their approximate masses, in kDa, and the domain start/end residue numbers are given, respectively, above and below each domain. The N-terminal mitochondrial targeting peptide that is removed upon internalization in the organelle is not represented. The domains are defined by their function (when known;?, function unknown), showing below each of them the corresponding acronym (ISD, GSD, BPSD, UFSD, CPSD and ASD) as defined in [2]. (B) Reactional steps of the CPS1 reaction, shown under the domains catalyzing them, colored as the domain. The thick empty horizontal black arrow denotes the migration of carbamate from the bicarbonate phosphorylation site to the carbamate phosphorylation site. (C) Stereo view of structural model of Cα trace of human CPS1 [42]. Each domain is colored differently and labeled in the same color. In the UFSD, the spheres mark the residues (numbered) hosting extremely drastic (red) or less drastic (blue) mutations found in CPS1D patients. Both ATP molecules and essential K^+ ions are placed in their sites by superimposition of the *E. coli* CPS structure (Protein Databank file 1BXR; [48]). (D) Relations of the UFSD with other domains. Two views of the domain (cartoon representation, in yellow) are shown, highlighting the residues hosting the mutations (red and blue spheres as above) illustrating that the mutations cluster at the regions of contact with the other domains (labeled). Only the parts of these other domains that contact the UFSD are shown, in surface representation, colored as in (A). In the right panel the ISD is shown as transparent surface to allow visualization of Ala949, which is clamped between the ISD, the GSD and the BPSD.

off when glutamate levels decrease, thus preventing excessive nitrogen draining by the urea cycle from an already low amino acid pool [18,19]. NAG activation is a unique property of CPS1 (and to a lesser extent of the piscine CPS1 homologue CPSIII) [20], not being shared by other CPSs, all of which are active in the absence of effectors and are insensitive to NAG [13,21,22]. NAG activation involves a functionally crucial cross-talk

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