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Journal of Genetics and Genomics 42 (2015) 249−260

JGG

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The Study of Carbamoyl Phosphate Synthetase 1 Deficiency Sheds Light on the Mechanism for Switching On/Off the Urea Cycle

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Received 31 October 2014; revised 22 March 2015; accepted 25 March 2015
Available online 1 April 2015

ABSTRACT

Carbamoyl phosphate synthetase 1 (CPS1) deficiency (CPS1D) is an inborn error of the urea cycle having autosomal (2q34) recessive inheritance that can cause hyperammonemia and neonatal death or mental retardation. We analyzed the effects on CPS1 activity, kinetic parameters and enzyme stability of missense mutations reported in patients with CPS1 deficiency that map in the 20-kDa C-terminal domain of the enzyme. This domain turns on or off the enzyme depending on whether the essential allosteric activator of CPS1, N-acetyl-L-glutamate (NAG), is bound or is not bound to it. To carry out the present studies, we exploited a novel system that allows the expression *in vitro* and the purification of human CPS1, thus permitting site-directed mutagenesis. These studies have clarified disease causation by individual mutations, identifying functionally important residues, and revealing that a number of mutations decrease the affinity of the enzyme for NAG. Patients with NAG affinity-decreasing mutations might benefit from NAG site saturation therapy with N-carbamyl-L-glutamate (a registered drug, the analog of NAG). Our results, together with additional present and prior site-directed mutagenesis data for other residues mapping in this domain, suggest an NAG-triggered conformational change in the β4-α4 loop of the C-terminal domain of this enzyme. This change might be an early event in the NAG activation process. Molecular dynamics simulations that were restrained according to the observed effects of the mutations are consistent with this hypothesis, providing further backing for this structurally plausible signaling mechanism by which NAG could trigger urea cycle activation *via* CPS1.

KEYWORDS: Urea cycle diseases; Inborn errors; Hyperammonemia; Site-directed mutagenesis; Restrained molecular dynamics; Allosteric regulation; Carbamoyl phosphate synthetase 1; Enzyme

INTRODUCTION

Carbamoyl phosphate synthetase 1 (CPS1) deficiency (CPS1D, OMIM #237300), a recessively inherited autosomal (2q34) (McReynolds et al., 1981) inborn error of the urea cycle (Freeman et al., 1964; Gelehrter and Snodgrass, 1974), has an estimated incidence of 1/50000 to 1/300000 (Uchino et al., 1998; Summar et al., 2013). CPS1 is the entry point of ammonia, the nitrogenous waste product of protein catabolism, into the urea cycle (Fig. 1A). Therefore, CPS1D causes pure hyperammonemia (Häberle and Rubio, 2014), leading to encephalopathy and even death (Brusilow and Horwich, 2001), and to depletion of downstream urea cycle intermediates, particularly of citrulline (Häberle and Rubio, 2014).

A large repertory of mutations affecting the *CPS1* gene (OMIM #608307; 201,425 nucleotides; start/end chromosome 2 coordinates, 211,342,405/211,543,830, plus strand; http://www.genecards.org/cgi-bin/carddisp.pl?gene=CPS1) has

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http://dx.doi.org/10.1016/j.jgg.2015.03.009
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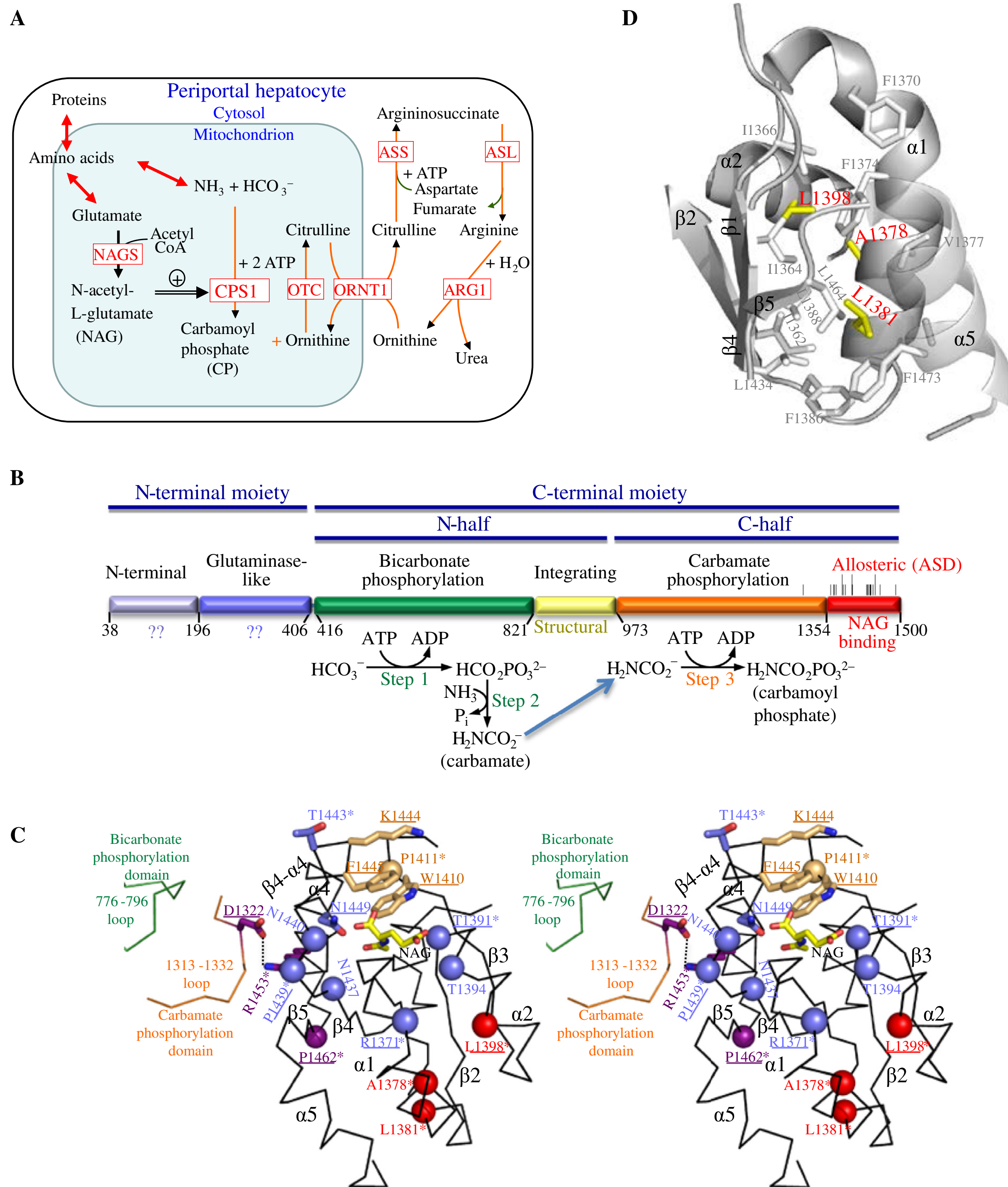

Fig. 1. The CPS1-NAG switch for urea cycle control.

A: Simplified view of the urea cycle to highlight its control at the level of CPS1. The double arrows denote bidirectional fluxes between the elements that are linked. Enzymes are boxed and abbreviated as follows: NAGS, NAG synthase; OTC, ornithine transcarbamylase; ASS, argininosuccinate synthetase; ASL, argininosuccinate lyase; ARG1, arginase 1. ORNT 1, ornithine/citrulline antiporter 1. For simplicity, not all products of the different reactions are indicated. The intramitochondrial part of the cycle, where the switch mechanism operates, is highlighted. **B:** Scheme of the mature CPS1 polypeptide (N-terminal mitochondrial targeting sequence removed), indicating its two moieties (top) that are homologous to the small and large subunits of *E. coli* CPS, the two halves of the large moiety (middle), and the domain composition (lower bar) with domain names above, domain boundaries given as residue numbers, and domain functions shown below ("??" means unknown function), including the domain localization of the three steps of the CPS1 reaction. The blue arrow indicates carbamate migration between both phosphorylation domains, a process that is unlikely to involve the integrating domain (Thoden et al., 1999). Vertical lines towards the C-end map CPS1D missense mutations and rationally-designed mutations analyzed here (listed in Tables 1 and 2). The longer lines indicate that two different mutations affect the same residue. **C:** Stereo view pair (two views distinguished by a 5° rotation around the vertical axis, to allow 3-D imaging with a stereoscopic viewer) of the crystallographic structure of the allosteric domain with NAG bound as previously modeled (Pekkala et al., 2009). Spheres and explicitly shown in amino acid side-chains mark residues hosting missense mutations that are discussed here and identified by labeling (residues underlined are those hosting mutations studied

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