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Reshaping the folding energy landscape of human carbonic anhydrase II by a single point genetic mutation Pro237His

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Abstract

Human carbonic anhydrase (HCA) II participates in a variety of important biological processes, and it has long been known that genetic mutations of HCA II are closely correlated to human disease. In this research, we investigated the effects of a genetic single point mutation P237, which is located on the surface of the molecule and does not participate in the HCA II catalysis, on HCA II activity, stability and folding. Spectroscopic studies revealed that the mutation caused more buried Trp residues to become accessible by solvent and caused the NMR signals to become less dispersed, but did not affect the secondary structure or the hydrophobic exposure of the protein. The mutant was less stable than the wild type enzyme against heat- and GdnHCl-induced inactivation, but its pH adaptation was similar to the wild type. The mutation slightly decreased the stability of the molten globular intermediate, but gradually affected the stability of the native state by a 10-fold reduction of the Gibbs free energy for the transition from the native state to the intermediate. This might have led to an accumulation of the aggregation-prone molten globular intermediate, which further trapped the proteins into the off-pathway aggregates during refolding and reduced the levels of active enzyme *in vivo*. The results herein suggested that the correct positioning of the long loop around P237 might be crucial to the folding of HCA II, particularly the formation of the active site.

Keywords: Protein aggregation; Human carbonic anhydrase II; Genetic mutation; Protein folding pathways; Molten globule

Abbreviations: ANS, 8-anilino-1-naphthalene sulfonic acid; CA, carbonic anhydrase; GdnHCl, guanidine hydrochloride; HCA, human carbonic anhydrase; CD, circular dichroism; I_{320}, intrinsic fluorescence intensity at 320 nm; I_{365}, intrinsic fluorescence intensity at 365 nm; IPTG, isopropyl-β,D-thiogalactopyranoside; *p*-NPA, *p*-nitrophenol acetate; SEC, size exclusion chromatography; WT, wild type.

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1. Introduction

Anfinsen's thermodynamic hypothesis, which is thought to be the central pillar of protein science, states that the amino acid sequence determines the native three-dimensional structure of a protein in a state of thermodynamic equilibrium corresponding to the system with the lowest free energy (Anfinsen, 1973). The folding and the stability of proteins in physiological conditions are known to be closely correlated to their functions, and thus aberrant protein folding/unfolding may cause loss-of-function of proteins and even disease (Dobson, 2001, 2003). Although the problem of how the folding is encoded in the sequence is not fully understood, it is known that some disease-related genetic mutations can gradually alter the folding or the stability of proteins (for example, Almstedt et al., 2004; Feng, Zhao, Zhou, & Yan, 2007; Francis, Berry, Moore, & Bhattacharya, 1999; Stefani & Dobson, 2003). Doubtless, knowledge of the biochemistry of disease-related mutations can provide invaluable information for understanding the mechanisms of these diseases and developing new therapies for them.

Human carbonic anhydrase (HCA) II, which belongs to a large zinc metalloenzyme family, catalyzes the reversible hydration of carbon dioxide (Lindskog, 1997). Its evolutionarily homologous forms have been found in most organisms from bacteria to human, and carbonic anhydrase (CA) appears to have an ancient lineage. As a model protein, the folding of CA has been well characterized as a multi-step process involving two intermediates (Bushmarina, Kuznetsova, Biktashev, Turoverov, & Uversky, 2001; Cleland & Wang, 1990; Henkens, Kitchell, Lottich, Stein, & Williams, 1982; Semisotnov et al., 1990; Wong & Tanford, 1973), in which the first but not the second refolding intermediate is prone to aggregate (Cleland & Wang, 1990; Jiang, Yan, & Zhou, 2006). As a vital enzyme for cell and body functions, CA participates in a variety of biological processes including respiration, calcification, acid–base balance, bone resorption, and the formation of aqueous humor, cerebrospinal fluid, saliva, and gastric acid (Dodgson, Tashian, Gross, & Carter, 1991). It has also long been known that genetic mutations of HCA II are closely correlated to several human diseases. About three decades ago, Sly et al. (1972) described three sisters with a form of osteopetrosis that was associated to HCA II mutation (Sly, Hewett-Emmett, Whyte, Yu, & Tashian, 1983). Since then, medical surveys have reported many CA II single point mutations such as Asn252Asp (Lin & Deutsch, 1972), Lys17Glu (Jones, Sofro, & Shaw, 1982), His107Tyr (Roth, Venta, Tashian, & Sly, 1992; Venta, Welty, Johnson, Sly, & Tashian, 1991), Tyr40Ter (Soda, Yukizane, Yoshida, Aramaki, & Kato, 1995), and the deletion of a single cytosine in codon 207 (Borthwick et al., 2003). Some of these mutations are thought to cause deficiencies and are regarded as the primary cause of an inherited syndrome of osteopetrosis with renal tubular acidosis and cerebral calcification (Hu, Lim, Ciccolella, Strisciuglio, & Sly, 1997; Sly & Hu, 1995). It has also been found that the disease-related mutations discovered in HCA II defects usually can be addressed as conformational diseases (Almstedt et al., 2004).

In 1983, one more single amino acid substitution Pro237His of HCA II (HCA II_{P237H}) was discovered in a Caucasian woman by screening of hemolysate (Jones & Shaw, 1983). This variant was reported to have similar enzyme activity but less heat stability when compared with the WT HCA II. However, only subjects heterozygous for this mutation have been found, and the lack of knowledge of how this mutation affects the structure and folding of HCA II makes it difficult to understand how this single point mutation leads to disease. According to the structure of HCA II (Hakansson, Carlsson, Svensson, & Liljas, 1992), Pro237 is located on the surface of the molecule and does not participate in the substrate binding or catalysis of the enzyme (Fig. 1). In this research, the P237H mutation was found to gradually destabilize the enzyme and affect the folding pathway of HCA II. These findings provide clues in understanding how this mutation causes loss-of-function folding disease *in vivo*. Moreover, the results herein also contribute to understanding the mechanisms of the folding of the model protein HCA II.

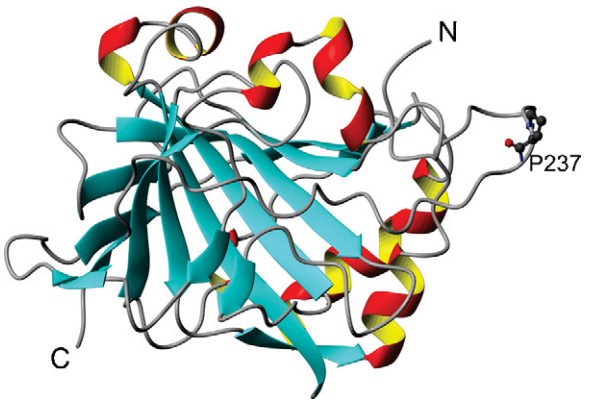

Fig. 1. Schematic structure of HCA II from PDB coordinates 2CBA. The location of P237 is highlighted by the ball-and-stick model. N and C denote the N- and C-terminus.