Single-residue variants of phenylalanine hydroxylase help to observe multiple structural isoforms that comprise the structural equilibrium

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Phenylalanine hydroxylase (PAH) catalyzes the hydroxylation of phenylalanine (Phe). In humans, Phe hydroxylation prevents its toxic accumulation. Persistent high Phe is the hallmark of the inborn error of metabolism, phenylketonuria (PKU). PKU is generally caused by dysfunctional PAH. Properly functioning PAH is attuned to Phe levels: elevated Phe allosterically activates PAH, while a drop in Phe returns PAH to its resting state. PKU-associated PAH is not properly tunable to changes in Phe levels, suggesting structural defects related to allosteric regulation. We have recently proposed that the structural basis of PKU lies in a disequilibrium of structural isoforms.1 Whereas decades of enzymology have elucidated the PAH catalytic mechanism, only recently was the first crystal structure of any full-length mammalian PAH reported.2 This rat PAH structure shows an auto-inhibited configuration, which is the resting-state form of PAH. Based on SEC-SAXS, that crystal structure differs significantly in inter-domain orientation from activated PAH.3 Two major challenges remain in determining the structural basis of PKU: (1) the activated form of PAH remains recalcitrant to crystallization, and (2) there are hundreds of PKU-associated missense variants, that occur throughout the entire length of the protein, and cause disease phenotypes that are not easily predicted on the basis of the resting state form of PAH structure. We will present: 1. the new hypothesis for the structural basis of PKU based on an equilibrium of multiple PAH structures;1 2. the first full-length structure of a human PAH, which was achieved by a single-residue substitution; and 3. our attempts at isolating activated PAH. Towards this end, we have designed single-residue variants that were predicted to change the balance of PAH structures. At least one of these variants is intrinsically and persistently activated; while its crystallization remains a challenge, it elucidates the value of an inter-domain interaction predicted to be important to stabilizing resting-state PAH.