

GABA Production by Glutamic Acid Decarboxylase is Regulated by a Dynamic Catalytic Loop

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Glutamic acid decarboxylase (GAD) is a pyridoxal 5'-phosphate (PLP) dependent enzyme present in all eukaryotes and in many prokaryotes. In mammals, isoforms of GAD, GAD65 and GAD67 catalyze the production of gamma-aminobutyric acid (GABA) from glutamate. GABA is an essential neurotransmitter inhibitor that controls processes such as neurogenesis, movement, tissue development, circadian clocks and blood glucose levels in humans. The two GAD isozymes function in concert to maintain an appropriate supply of GABA. Specifically, GAD67 is constitutively active and is responsible for basal GABA production. In contrast, GAD65, a known autoantigen in type I diabetes and rare brain disorders, is transiently activated in response to the demand for extra GABA by cycling between an active holo-form (cofactor bound) and an inactive apo-form (without the cofactor). The structural basis for regulation of GAD65 activity is unknown.

We have determined the X-ray crystal structures of both GAD isoforms at 2.1Å resolution. The structure of GAD67 reveals a tethered loop covering the active site, providing a catalytic environment that sustains GABA production. In contrast, the structure of GAD65 revealed that the same catalytic loop was inherently mobile. Kinetic and mutagenesis studies suggest that mobility in the catalytic loop in GAD65 permits a side reaction that results in cofactor release and GAD65 auto-inactivation. Together, these data show that the dynamics of the GAD catalytic loop regulates GABA homeostasis and reveal the structural basis for the crucial role of each GAD isoform in mammals.