Self-activation and oligomeric structure of glutamate synthase

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Glutamate synthase (GltS) catalyzes the reductive transfer of L-glutamine amide group to the C(2) carbon of 2-oxoglutarate (2-OG) producing two molecules of L-glutamate. GltS fall in one of three classes (the bacterial NADPH-dependent GltS, the plant-type ferredoxin-dependent form, and the eukaryotic NADH-dependent enzyme), which share a homologous subunit (the α subunit of NADPH-GltS (αGltS), the single polypeptide chain of Fd-GltS and the *N*-terminal 3/4th of the NADH-GltS) where the glutamine-dependent glutamate synthesis takes place. In the NAD(P)H-GltS forms, a second subunit (the NADPH-GltS β subunit or the *C*-terminal 1/4th of the NADH-GltS) is present and it catalyzes NAD(P)H oxidation and the transfer of the reducing equivalents to the site of reductive glutamate synthesis on αGltS.

Among peculiar features of GltS are: *(i)* the tight control of glutamine hydrolysis (at the glutaminase site in the N-terminal amidotransferase domain of αGltS or in the corresponding regions of the other two GltS forms), which takes place only when 2-OG and reducing equivalents are present at the synthase site of the same subunit; *(ii)* the coupling of glutamine hydrolysis with glutamate synthesis from 2-OG and the ammonia molecule released from glutamine and transferred from the glutaminase to the synthase site so that the stoichiometry of glutamine hydrolyzed and glutamate formed from 2-OG is strictly 1:1. In NADPH-GltS such a tight control requires the integrity of the αβ protomer being partially lost in the isolated αGltS. Furthermore, the presence of reduced Fd bound to the reduced enzyme is essential in order to obtain the catalytically active Fd-GltS form.

The characterization of protein variants carrying substitutions of amino acyl residues that structural and modeling studies suggested to play key roles in cofactor binding and in the cross-regulation of the catalytic subsites of GltS is contributing to the elucidation of the structure and regulation of this enzyme and is highlighting features common to other amidotransferases. In the absence of crystals of the NADPH-GltS αβ holoenzyme or of the Fd/Fd-GltS complex, solution and single molecule structural approaches have been used to obtain information on the stoichiometry of the Fd/Fd-GltS complex and on the oligomerization state of the NADPH-GltS. A 9.6 Å model of the $(\alpha\beta)_6$ hexameric complex of NADPH-GltS, which has been obtained by combining cryo-electron microscopy and small-angle X-ray scattering will be presented and its functional implications will be discussed.