ESRF	**Experiment title:** Crystal structure of glutamate synthase, a complex iron-sulfur flavoprotein	**Experiment number**: LS-1664
Beamline: BM14	**Date of experiment**: from: 17.02.2000 to:19.02.2000	**Date of report**: 1 August 2000
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1. Introduction

Nitrogen is the second most abundant element and is a critical nutrient for plant and microorganism growth and yield. The improvement in nitrogen use by plants requires a thorough knowledge of the genes and enzymes that control primary and secondary ammonia assimilation. One of the key enzymes in this process is the iron-sulfur flavoprotein glutamate synthase (GltS). This enzyme catalyzes the reductive transfer of the amide group of L-glutamine to 2-oxoglutarate, producing two molecules of L-glutamate. The reducing equivalent are provided by NADH, NADPH or reduced ferredoxin (for reviews see 1, 2):

$$\text{L-glutamine} + \text{2-oxoglutarate} + 2e^- + 2H^+ \leftrightarrow 2\ \text{L-glutamate}$$

GltS is found in all types of organisms and its gene and amino acid sequence is remarkably well conserved. On the basis of primary structures and known biochemical properties three different classes of glutamate synthases are distinguished:

***NADPH-glutamate synthase. Bacterial GltS is specific for NADPH and comprises two subunits with a large α subunit of about 150 kDa and a smaller β subunit of about 50 kDa that form an active protomer containing two flavins cofactors and three Fe/S clusters.**

***Ferredoxin-dependent glutamate synthase. Plants and cyanobacteria contain a ferredoxin-dependent GltS. This enzyme is composed of only one subunit of about 150 kDa, similar to the α subunit of NADPH-GltS, and contains one flavin and a one or two Fe/S clusters.**

***NADH-glutamate synthase. Plants, lower animals, fungi and yeasts contain a NADH-dependent GltS. The enzyme is composed of a single subunit of about 200 kDa, which is derived from a fusion of the two subunits of NADPH-GltS.**

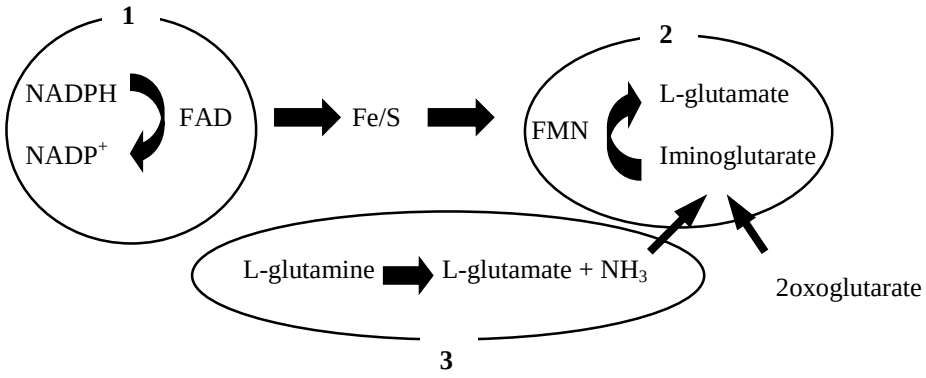

Figure 1: Reaction scheme and cofactor distribution in NADPH-GltS

2. Glutamate synthase from *A. brasilense*

The NADPH-dependent Glts from the nitrogen fixing bacterium *Azospirillum brasilense* is one of the most well characterized glutamate synthases. The enzyme is a tetramer of αβ heterodimers, each containing three iron-sulphur clusters, a molecule of FMN and one of FAD. Biochemical studies in combination with the recent structural data (see below) have shown that this GltS reacts via three distinct catalytic sites (2). The smaller 52 kDa subunit contains site 1 whereas the larger 162 kDa subunit contains sites 2 and 3 (Fig. 1). The catalytic cycle involves two electron reduction of the FAD by NADPH (site 1), followed by electron transfer to the FMN (site 2) through the iron-sulphur clusters. Next, this cofactor reduces the iminoglutarate formed by addition onto 2-oxoglutarate of the ammonia resulting from Gln hydrolysis (site 3). Thus, GltS is a truly remarkable catalyst, able to employ the electron transfer from an "entry site" (the FAD reduced by NADPH) to an "exit site" (the FMN) as driving force for the hydrolysis of Gln by a glutamine amidotransferase active centre. In this way, the directionality in the electron flow makes the enzyme-catalyzed reaction essentially irreversible.

The functional core of the enzyme is formed by the α subunit, which carries sites 2 and 3. Indeed, this subunit displays glutamate synthase activity using an artificial electron donor, such as dithionite (3).

3. MAD structure determination at BM14 and ID14-2

We have determined the crystal structure of the large α subunit of GltS from *A. brasilense*. The subunit consists of 1479 amino acids, a FMN molecule and a 3Fe-4S cluster. The crystals used for structure determination belong to space group $P3_121$ with unit cell parameters a=b=234 Å and c=304 Å. A MAD experiment based on the presence of the 3Fe-4S clusters was carried out at BM14 (see Table 1). MAD data were measured to 4.3 Å. The Patterson function calculated with the anomalous differences at 6.0 Å resolution was examined using the program SHELX. The positions of two clusters were identified, suggesting that the asymmetric unit contained two subunits, each of them carrying a 3Fe-4S,

Table 1: Summary of Data Collection

	Native	MAD		
		Fe peak	Fe inflection p.	Fe remote
		λ=1.734 Å	λ=1.741 Å	λ=0.9918 Å
Beamline	ID14-EH1	BM14	BM14	BM14
Max resolution	3.0 Å	4.3 Å	4.3 Å	4.3 Å
Observations	758,585	335,184	329,402	316,594
Unique reflections	184,182	65,076	65,900	65,865
R_{merge}	6.4 (1.2)	5.7 (3.2)	5.5 (3.0)	7.0 (6.1)
(%)	9.2 (45.6)	10.6 (20.6)	10.5 (20.8)	8.1 (10.3)
Multiplicity	4.1	5.2	5.0	4.8
Completeness(%)	98.6 (94.1)	99.9 (99.1)	99.9 (99.8)	99.9 (99.7)
Anom. Compl.(%)	-	97.9 (91.0)	97.8 (90.2)	96.1 (81.2)
R_{Cullis}		0.97	-	0.94
Phasing power				
(centric/acentric)		0.29/0.39	-	0.34/0.51
Figure of Merit			0.17 (0.08)	

cluster with a large 78% solvent content. Thus, it was possible to measure the anomalous signal of *six Fe atoms out of a total of about 22000"light" atoms present in the asymmetric unit.*

The 4.3 Å MAD phases were extended to 3.0 Å by solvent flattening and twofold averaging. For this purpose, a native data set collected on ID14-EH1 (see progress report submitted in February 2000) was employed. The resulting electron density map was of good quality and allowed the building of the whole model. After maximum likelihood refinement, the R-factor and free R-factor for the current model are 25.8% and 28.7% for 184,349 and 1,835 reflections, respectively.

4. <u>The overall structure of the α subunit of GltS</u>

The structure of the α subunit of *A. brasilense* GltS consists of four well-defined domains. The N-terminal domain (residues 1-422) is topologically similar to the so-called class I amidotransferases. (4). An N-terminal Cys residue acts as the nucleophile which catalyses the hydrolysis of Gln to produce ammonia. The other catalytic domain is formed by residues 780-1183 and binds FMN and the 3Fe-4S cluster. This domain is responsible for the uptake of the electrons from the electron donor and for the flavin-dependent reduction of the iminoglutarate intermediate (Fig. 1). The α-subunit contains two further domains, which do not have a known catalytic function. The central domain (residues 423-779) has a α/β topology and is likely to have a key role in the communication between active centers whereas the C-terminal domain (1184-1472) has a right-handed β-helix topology.

A prominent feature revealed by the three-dimensional structure is a 27 Å tunnel which goes across the protein and connects the Gln site on the N-terminal domain (site 3 in Fig. 1) to the 2-oxoglutarate binding site in proximity of the FMN (site 2 in Fig. 1). Such a tunnel is functionally crucial in that it allows diffusion of the ammonia resulting from Gln hydrolysis to the 2-oxoglurate to generate the iminoglutarate intermediate. The tunnel is lined by two

extended stretches of polypeptide chains which are likely to have a critical role in the coordination of the functioning of the various catalytic and redox sites present in the protein.

5. References

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