ESRF

ESRF	**Experiment title:** Structural Investigation of 3-methyl aspartate ammonia lyase.	**Experiment number**: LS 1590
Beamline: BM30A	**Date of experiment**: from: 30/10/99 to: 1/11/99	**Date of report**: July 2003
Shifts: 6	**Local contact(s)**: Dr. M.Roth	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

Prof. D.W. RICE
Dr. P.J. ARTYMIUK
Dr. PATRICK J. BAKER*
Dr J.B. RAFFERTY*

University of Sheffield, Department of Molecular Biology and Biotechnology, Sheffield, S10 2TN. U.K.

Report:

Insights into Enzyme Evolution Revealed by the Structure of Methylaspartate Ammonia Lyase

Structure **10(1)** *105-11,* 2002.

C. W. Levy, P. A. Buckley, S. Sedelnikova, Y. Kato, Y. Asano, D. W. Rice and P. J. Baker

Abstract

Methylaspartate ammonia lyase (MAL) catalyzes the magnesium-dependent reversible ,αβ-elimination of ammonia from L-*threo*-(2S,3S)-3-methylaspartic acid to mesaconic acid. The 1.3 Å MAD crystal structure of the dimeric *Citrobacter amalonaticus* MAL shows that each subunit comprises two domains, one of which adopts the classical TIM barrel fold, with the active site at the C-terminal end of the barrel. Despite very low sequence similarity, the structure of MAL is closely related to those of representative members of the enolase superfamily, indicating that the mechanism of MAL involves the initial abstraction of a proton α to the 3-carboxyl of (2S,3S)-3-methylasparic acid to yield an enolic intermediate. This analysis resolves the conflict that had linked MAL to the histidine and phenylalanine ammonia lyase family of enzymes.

Structural Analysis of *Bacillus subtilis* SPP1 Phage Helicase Loader Protein G*39*P

J. Biol. Chem. **278** 15304-15312, 2003.

Scott Bailey, Svetlana E. Sedelnikova, Pablo Mesa, Sylvia Ayora, Jon P. Waltho, Alison E. Ashcroft, Andrew J. Baron, Juan C. Alonso, and John B. Rafferty

Abstract

The *Bacillus subtilis* SPP1 phage-encoded protein G*39*P is a loader and inhibitor of the phage G*40*P replicative helicase involved in the initiation of DNA replication. We have carried out a full x-ray crystallographic and preliminary NMR analysis of G*39*P and functional studies of the protein, including assays for helicase binding by a number of truncated mutant forms, in an effort to improve our understanding of how it both interacts with the helicase and with the phage replisome organizer, G*38*P. Our structural analyses reveal that G*39*P has a completely unexpected bipartite structure comprising a folded N-terminal domain and an essentially unfolded C-terminal domain. Although G*39*P has been shown to bind its G*40*P target with a 6:6 stoichiometry, our crystal structure and other biophysical characterization data reveal that the protein probably exists predominantly as a monomer in solution. The G*39*P protein is proteolytically sensitive, and our binding assays show that the C-terminal domain is essential for helicase interaction and that removal of just the 14 C-terminal residues abolishes interaction with the helicase *in vitro*. We propose a number of possible scenarios in which the flexibility of the C-terminal domain of G*39*P and its proteolytic sensitivity may have important roles for the function of G*39*P *in vivo* that are consistent with other data on SPP1 phage DNA replication.