Structural and mechanistic studies on N2-(2-carboxyethyl)arginine synthase

M. E. C. Caines, K. E. McAuley[1] and C. J. Schofield

Chemistry Research Laboratory, University of Oxford, Mansfield Road, OX1 3TA, Oxford, UK
[1]Diamond Light Source, Chilton, OX11 0DE, Didcot, UK

The first step in the biosynthesis of the clinically important β-lactamase inhibitor, clavulanic acid, involves condensation of two primary metabolites, D-glyceraldehyde-3-phosphate (D-G3P) and L-arginine, to give N^2-(2-carboxyethyl)arginine (CEA), a β-amino acid. This unusual N-C bond forming reaction is catalysed by the thiamin diphosphate (ThDP) dependent enzyme N^2-(2-carboxyethyl)arginine synthase (CEAS).

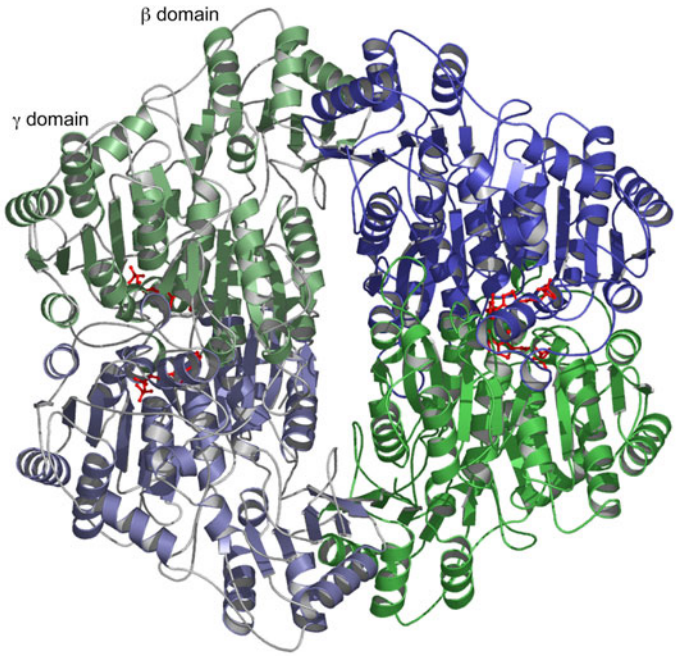

Figure 1. Tetrameric structure of CEAS.

The structure of CEAS has been solved to 2.35 Å resolution in two space groups, $P2_12_12_1$ and $P2_12_12$. In both, the enzyme was observed in a tetrameric form (Figure 1), composed of a dimer of two more tightly associated dimers, consistent with both mass spectrometric and gel filtration chromatography studies. Both ThDP and Mg^{2+} cofactors were present at the active site, with the ThDP adopting a 'V' conformation as observed in related enzymes.

In the original structure, a sulfate anion was observed bound in the active site of the enzyme in a location proposed as a binding site for the phosphate group of the D-G3P substrate. A number of mechanistic implications (Figure 2) of the active site arrangement were elucidated, including the potential role of the aminopyrimidine ring of the ThDP.

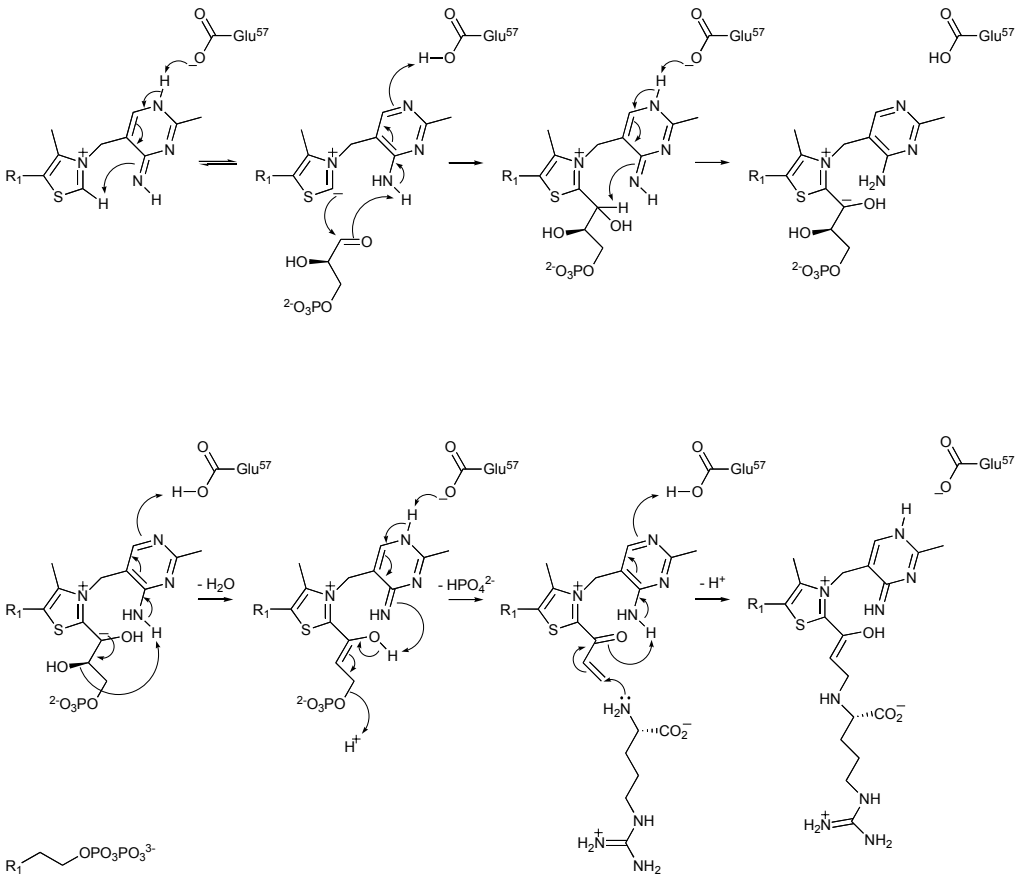

Figure 2. Proposed outline mechanism of CEAS.

The structural information obtained in these studies will be useful in protein engineering experiments aimed at the production of alternative β-amino acids and derivatives of clavulanic acid.