Structure of Thi1: Thiamin Biosynthesis in *Arabdopsis thaliana*

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Thiamin is an essential coenzyme in all living organisms. It is formed in two biosynthetic routes, one for the synthesis of 2-methyl-4-amino-5-hydroxymethylpyrimidine pyrophosphate (the pyrimidine moiety) and another for the synthesis of 4-methyl-5-(b-hydroxyethyl)thiazole phosphate (the thiazole moiety). Here we describe the three-dimensional structure of Thi1, the only known thiazole biosynthetic enzyme in Eukaryotes, bound to 2-carboxylate-4-methyl-5-b-(ethyl adenosine 5'-diphosphate) thiazole. This intermediate has not been described before and was either formed by the enzyme during the Thi1 heterologous expression in *E. coli* or was sequestered from the bacterial cytoplasm. Based on the structure, we putatively propose that Thi1 is the thiazole synthase from *Arabidopsis thaliana*

Crystals grow as thin plates (0.1 x 0.1 x 0.01 mm) by vapor diffusion with well solution of 100 mM MES pH 6.0, 40% (v/v) MPD and 1.5% (w/v) heptane-1,2,3-triol. Crystals belong to space group F222 with unit cell dimensions of a=102.356 Å, b=133.147 Å and c=142.301Å with two molecules in the asymmetric unit. X-ray fluorescence scan at beamline ID29 of ESRF revealed an unforeseen bound Zn ion which allowed the structure solution by SAD phasing. The final refinement to 1.6 Å includes the one Zn ion and one ligand per monomer and a total of 438 water molecules, with final Rfactor 13.9% and Rfree 17.1%.

Keywords: thiamin biosynthesis, SAD, protein structure