

Structural study of methylenetetrahydrofolate reductase from *Pseudomonas aeruginosa*.

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Methylenetetrahydrofolate reductase (MetF) is an important enzyme that catalyzes the reduction of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, which is required for the conversion of L-homocysteine to L-methionine [1, 2]. This process is involved in the regulation of methylation reactions that play a crucial role in bacterial defense systems and metabolism [3]. Inhibition of this enzyme can lead to severe methylation disorders, potentially increasing bacterial susceptibility to antibiotics. As the sensitivity of *Pseudomonas aeruginosa* to antibiotics and disinfectants continues to decline, infections caused by this pathogen are becoming increasingly difficult to treat and pose a significant health threat, particularly to immunocompromised patients [4].

To address this problem, we undertook structural studies of this enzyme as part of a structure-based drug design approach. The enzyme was expressed in an *Escherichia coli* system, purified and crystallized. The initial crystals diffracted X-rays to a resolution of 2.7 Å; however, the crystal structure could not be refined satisfactorily, with R/Rfree parameters 0.29/0.36. A careful analysis of the diffraction dataset indicated a resolution anisotropy across three directions (ranging from 3.0 to 1.8 Å), as well as significant intensity fluctuations at specific orientations, most probably caused by a crystal slippage. The crystallographic pathologies mentioned above are probable reasons why data processing with XDS [5] did not yield satisfactory results. To overcome problems with diffraction data analysis, the data were processed using HKL software [6] with an automatic correction option. As a result, the final data set was processed to the ultimate resolution of 1.95 Å and allowed to refine the crystal structure to the reasonable R/Rfree parameters equal to 0.20/0.24, enabling this project to move to the next stage of research - ligand screening.

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