The crystal structure and slow time-resolved oxidative decay of an *E. coli* DHFR complex with tetrahydrofolate with implications to drug design

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Dihydrofolate reductase (DHFR) catalyzes the stereospecific reduction of 7,8-dihydrofolate (FH2) to (6s)-5,6,7,8-tetrahydrofolate (FH4) via hydride transfer from NADPH. The consensus *Escherichia coli* DHFR mechanism involves conformational changes between closed and occluded states occurring during the rate-limiting product release step. To the best of our knowledge, we determined the first crystal structure of an *E. coli.* DHFR:FH4 rate-limiting product release complex in an occluded conformation at 1.03 Å resolution. We also determined another occluded complex structure of *E. coli* DHFR with a slow-onset nanomolar inhibitor that contrasts with the methotrexate complex. These structures suggest a plausible strategy for designing DHFR antibiotics by targeting FH4 product conformations to combat emerging drug resistance. We also uncovered via time-resolved crystallography a putative FH4-FH2 intermediate that features a near coplanar geometry of the bicyclic pterin moiety and a tetrahedral sp^3 C6 geometry. This unusually slow reverse oxidative decay involves a C6 sp^3 to sp^2 chemical transition at room temperature *in crystalline* with an estimated half-life of 2-3 days.

References

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