The Crystal Structure of Dihydrofolate Reductase-Thymidylate Synthase from Cryptosporidium hominis Reveals a Novel Architecture for the Bifunctional Enzyme

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Cryptosporidium hominis is an emerging pathogen that primarily affects immune-compromised patients, including those with AIDS. There is no effective cure at this point for the degenerative wasting disease that can follow infection. Dihydrofolate reductase-thymidylate synthase (DHFR-TS) is a bifunctional enzyme in C. hominis and is crucial in the folate cycle for the production of dTMP, one of the four DNA bases. DHFR-TS is an excellent drug target since its inhibition leads to the death of the pathogen. In apicomplexan protozoa, including C. hominis, the gene for DHFR-TS can be partitioned into a DHFR domain, a linker domain and a TS domain. In human cells, DHFR and TS are separate, monofunctional enzymes.

We have determined the X-ray crystal structure of DHFR-TS from C. hominis. The structure reveals that the linker polypeptide between the DHFR and TS domains has important structural interactions with the opposite monomer of the homodimeric enzyme. A comparison of the structure of DHFR-TS from C. hominis and structures of DHFR-TS from Plasmodium falciparum [11] and Leishmania major [5] shows that there are significant structural differences between the apicomplexan and kinetoplastid forms of the enzyme. Additionally, the DHFR and TS domains of the C. hominis enzyme have important sequence and structural differences from the human forms of the enzymes. The species-specific differences are important for future inhibitor design targeting C. hominis.

RESULTS AND DISCUSSION

The crystal structure of DHFR-TS from C. hominis (ChDHFR-TS) reveals that the protein is a homodimer and forms the canonical TS dimer interface (Fig. 1). The two DHFR domains sit on the “shoulders” of the TS monomers. A two-fold non-crystallographic symmetry axis relates the two monomers of the protein. The ChDHFR-TS protein fold is unique. The ChDHFR-TS polypeptide forms the DHFR domain, crosses to the opposite monomer of the homodimeric enzyme, forms an 11-residue helix that interacts with the DHFR active site of the opposite monomer, and then crosses back and forms the TS domain. In general, the structures of the C. hominis DHFR and TS domains resemble other known structures of DHFR and TS, although there are some specific residue and loop differences that may prove to be important for species-specific drug design.

The residue differences between DHFR-TS from C. hominis and C. parvum, the bovine isolate of the parasite, are in regions that are seemingly insignificant to catalysis. Seven of the ten residue substitutions are in primarily surface-exposed residues. Two are in the second shell of residues away from the active site, in the interior of the protein and close to the linker domain that crosses between monomers. The last residue substitution is directly in the middle of the linker domain where the polypeptide crosses. The lack of significant structural differences or residue substitutions in catalytically important regions between the DHFR-TS proteins of C. hominis and C. parvum explains the similarities in IC50 values observed with several DHFR inhibitors [7].

The structure of ChDHFR-TS is similar in many respects to the structure of DHFR-TS from Plasmodium falciparum (PfDHFR-TS) [11], but both of the apicomplexan DHFR-TS proteins differ significantly from the structure of DHFR-TS from the kinetoplastid, Leishmania major (LmDHFR-TS) [5]. Both ChDHFR-TS and PdDHFR-TS have long linker regions between the DHFR and TS domains (58 and 89 residues, respectively). Both structures have helices, donated from the opposite monomer, located at the active site of DHFR. In both cases, the polypeptide chain then returns to form the TS domain. In the PdDHFR-TS structure, however, the electron density for the linker region was not clear and only the helix and connection back to TS were modeled. In ChDHFR-TS, this entire region was visible in the electron density and was modeled.

In stark contrast, however, the LmDHFR-TS protein has a very short (two-residue) linker between the DHFR and TS domains. The taut tether restricts the orientation of DHFR relative to TS. As a result, the LmDHFR domain is rotated approximately 180 degrees relative to the ChDHFR domain. The DHFR active site in ChDHFR-TS points toward the cavity between the two DHFR monomers and the DHFR active site in LmDHFR-TS points in the opposite direction, toward the outside of the molecule. Therefore, in LmDHFR-TS, the DHFR and TS active sites are on the same side of the monomer whereas in ChDHFR-TS, they are on opposite sides of the monomer. Given the structural differences between the apicomplexan and Leishmania forms of DHFR-TS, it is important, especially when modeling any compounds that are located outside the active site, to use a protein template that correlates with the species of interest.

In the DHFR active site, dihydrofolate and NADPH are bound using several of the canonical interactions seen in other structures of...
In conclusion, the crystal structure of ChDHFR-TS reveals a new fold for DHFR-TS in which each polypeptide chain of the homodimeric enzyme is associated with both monomers. There are few significant residue substitutions between DHFR-TS of C. hominis and C. parvum, allowing the successes of drug design for DHFR-TS from C. hominis to extend to C. parvum. Finally, there are important residue substitutions between C. hominis and human DHFR and TS that can be used in the design of species-selective inhibitors.

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