Structure-Activity Relationships for a Series of m-Substituted Pyrimethames *versus E. coli* DHFR

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Pyrimethamine and many of its derivatives are inhibitors of the enzyme dihydrofolate reductase (DHFR). Interactions between the compounds I–IV and *E. coli* dihydrofolate reductase have been investigated by means of molecular mechanics, molecular orbital calculations and docking studies.

Modeling of each of these structures began using the crystal structure of m-azidopyrimethamine (MZP) and the geometry was optimized by means of MNDO calculations in MOPAC. Partial atomic charges used were those from this calculation.

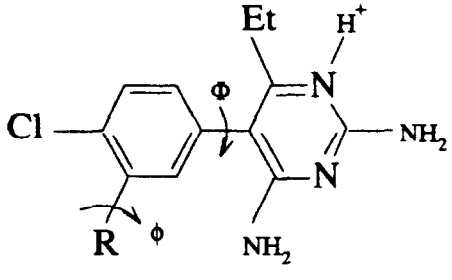
MZP was docked into DHFR using the HYDRA program, water molecules being removed where unacceptable contacts were made. The interaction energy between enzyme and substrate was monitored against the position of the inhibitor. A low energy conformation of MZP with the expected hydrogen bonding pattern was used as a starting point for docking the other three molecules.

The angle of twist between the two rings is obviously important, and for the pyrimethamine studies, this angle was varied from $-60°$ to $+60°$. However, the substituted pyrimethamines II–IV form two distinct rotamers, so both of these were considered in each case. They have been designated *cis* and *trans* according to the orientation of the substituent in relation to the 6-ethyl group.

All interaction energies were found to be negative, apart from cis MZP. In all the substituted compounds, the trans isomer is found to bind more strongly than the cis, and, in fact, trans MZP has the lowest interaction energy of -119 kcal mol^{-1}. The greater affinity of the trans isomers for DHFR appears to be due to the presence of a hydrophobic pocket in the active site bound by residues ile14, gly15 and met16, which could accommodate a bulky substituent in the meta position.

It was found that only twist angles of between 60° and 90° in pyrimethamine gave reasonable bound conformations; increasing the angle beyond 90° produced unacceptably high energies.

The interaction energies for this series of complexes have been compared with the results of assays on *E. coli* and rat liver DHFR. There is an apparent discrepancy in the case of MAP, but this may be due to the increased polarity of the molecule due to the NH_2 group leading to an increased affinity for the solvent.



compound	R
I	H pyrimethamine
II	NHz m-aminopyrimethamine
III	Nz m-azidopyrimethamine
IV	NOz m-nitropyrimethamine

Theoretical Study of Dielectric Constant of Protein

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Dielectric properties of a protein molecule were examined by defining and calculating a "local dielectric constant" with the aid of the normal mode analysis. This local dielectric constant was composed of the electronic polarization of atoms and of the orientational polarization of local dipoles in a protein. The former was approximated by a summation of atomic polarizations of the whole atoms in a protein molecule. The latter was calculated from the fluctuation of the local dipole based upon the fluctuation-dissipation theorem. The degree of the dipole fluctuation was measured from the positional fluctuation of each atom that can be observed by the normal mode analysis. The local dielectric constant at the deep inner of the protein was about 2.5–3, which was mainly due to the electronic polarization. However, the local dielectric constant was much larger than 3 around the atoms of peptide dipoles, the positional fluctuation of which was large. Assuming a minimum volume for a continuum model, the local dielectric constant was sometimes larger than 10 in such cases. The distribution of the local dielectric constant and the dielectric environment of each protein atom were illustrated on the computer graphics screen by color codes.

Using a set-of-bricks model representing a protein molecule, the local dielectric constant of each small cube was calculated by the present method. Electrostatic potential of the protein-solvent system was then numerically calculated by solving Poisson and Poisson-Boltzmann equations[1] based upon these locally defined inhomogeneous dielectric constants instead of the previous uniform dielectric model.

1 Nakamura, H. and Nishida, S. *J. Phys. Soc. Jpn.* 1987, **56**, 1609–1622