Effect of an enzyme binding site on the electronic distribution of bound inhibitors: 2,4-diaminopyrimidine in dihydrofolate reductase

Alastair F Cuthbertson

Chemistry Department, Purdie Building, University of St Andrews, Fife KY16 9ST, UK

Graphical displays of the electrostatic potential of 2,4-diaminopyrimidine both isolated and incorporated into the binding site of the enzyme dihydrofolate reductase are used to show how little polarization of the inhibitor is caused by the enzyme.

Keywords: enzymes, binding sites, graphics

received 13 May 1985, accepted 10 June 1985

In order to investigate how an enzyme perturbs the electron distribution of bound inhibitors, we have studied the effects of dihydrofolate reductase on diamino pyrimidine rings.

The enzyme dihydrofolate reductase is essential for cell synthesis and blockade of its activity leads to drugs that have anti-cancer properties. We have shown previously that the binding enthalpy of the drug methotrexate to the enzyme dihydrofolate reductase can be computed in an *ab initio* quantum mechanical fashion[1], using the Hayes and Kollman partial point charge approximation[2-3]. The atoms of the amino acid residues in the binding site are replaced by appropriate point charges calculated previously. As long as the residues have the correct overall charge, and several hundred atoms are included, then individual values seem not to be too critical.

Other *ab initio* studies of the binding energy of the pteridine fragment of methotrexate (see Figure 1) have been published[4], but these used simplified models of the enzyme: formate was used to represent aspartate, and only a few of the residues were considered. In the method used previously in this laboratory, the full binding site has been used in the computation[1].

The calculation of the binding energy alone, although useful, gives no real insight into why certain molecules bind better than others. To this aim, we have calculated

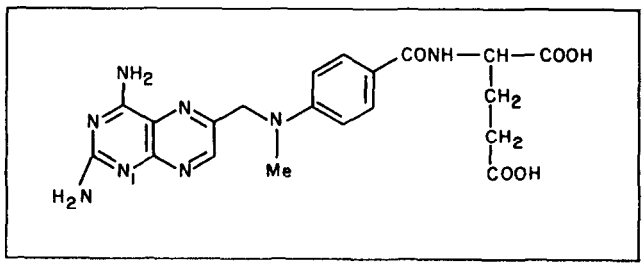


Figure 1. The drug molecule methotrexate with the atom labelling scheme

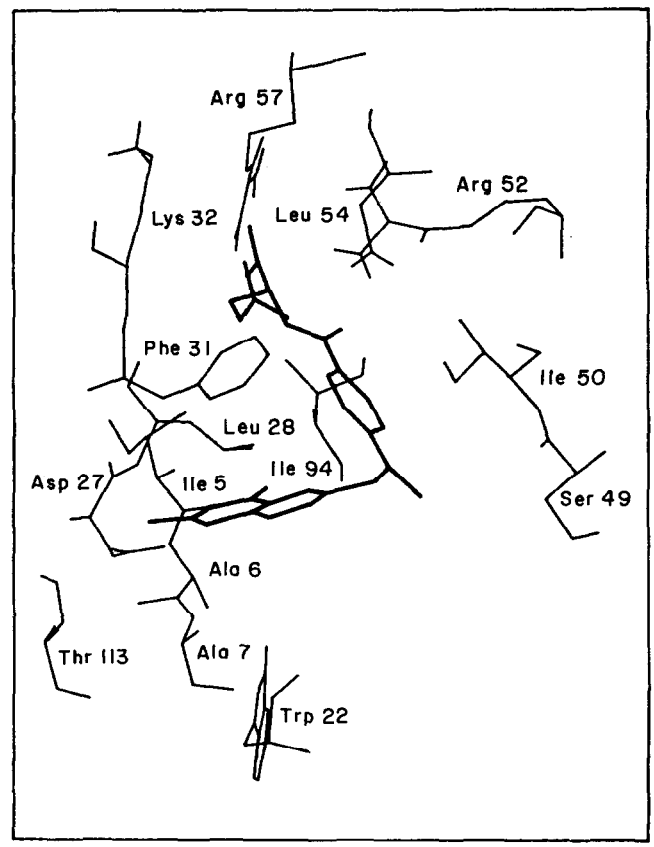


Figure 2. The binding site of E. coli *dihydrofolate reductase with methotrexate (bold lines). Plot produced on an ICL Perq computer using 'Display 3'*

 0263-7855/85/040131-04 $03.00

the electron density, electrostatic potential and Mulliken population analyses of 2,4-diaminopyrimidine, which is one of the rings present in the pteridine fragment of methotrexate and also the ring in trimethoprim. Rings, both protonated and unprotonated at N1 and perturbed and unperturbed by the partial charges of the binding site, were studied.

It is known from UV spectroscopy[5] that methotrexate and trimethoprim bind to the enzyme in a protonated form, and the calculation of the binding enthalpy by the partial charge method reflects this. However, it must be remembered that this estimates the charge–charge interaction *in vacuo*, and the actual values obtained are too high by a factor of ten[6].

METHOD AND RESULTS

All calculations were made with the Gaussian 70 program[7] modified to accept partial charge input and implemented on an ICL 2988 computer. The density matrices obtained from the computations were fed into the Denpot program[8] which calculated the electron density and electrostatic potential in the plane of the ring with a grid spacing of 0.4 A. The binding site of *E. coli* dihydrofolate reductase consists of 15 residues Ile 5, Ala 6, Ala 7, Trp 22, Asp 27, Leu 28, Phe 31, Lys 32, Ser 49, Ile 50, Arg 52, Leu 54, Arg 57, Ile 94 and Thr 113[9]: the overall charge is +2. Figure 2 shows the binding site of *E. coli* dihydrofolate reductase with the drug

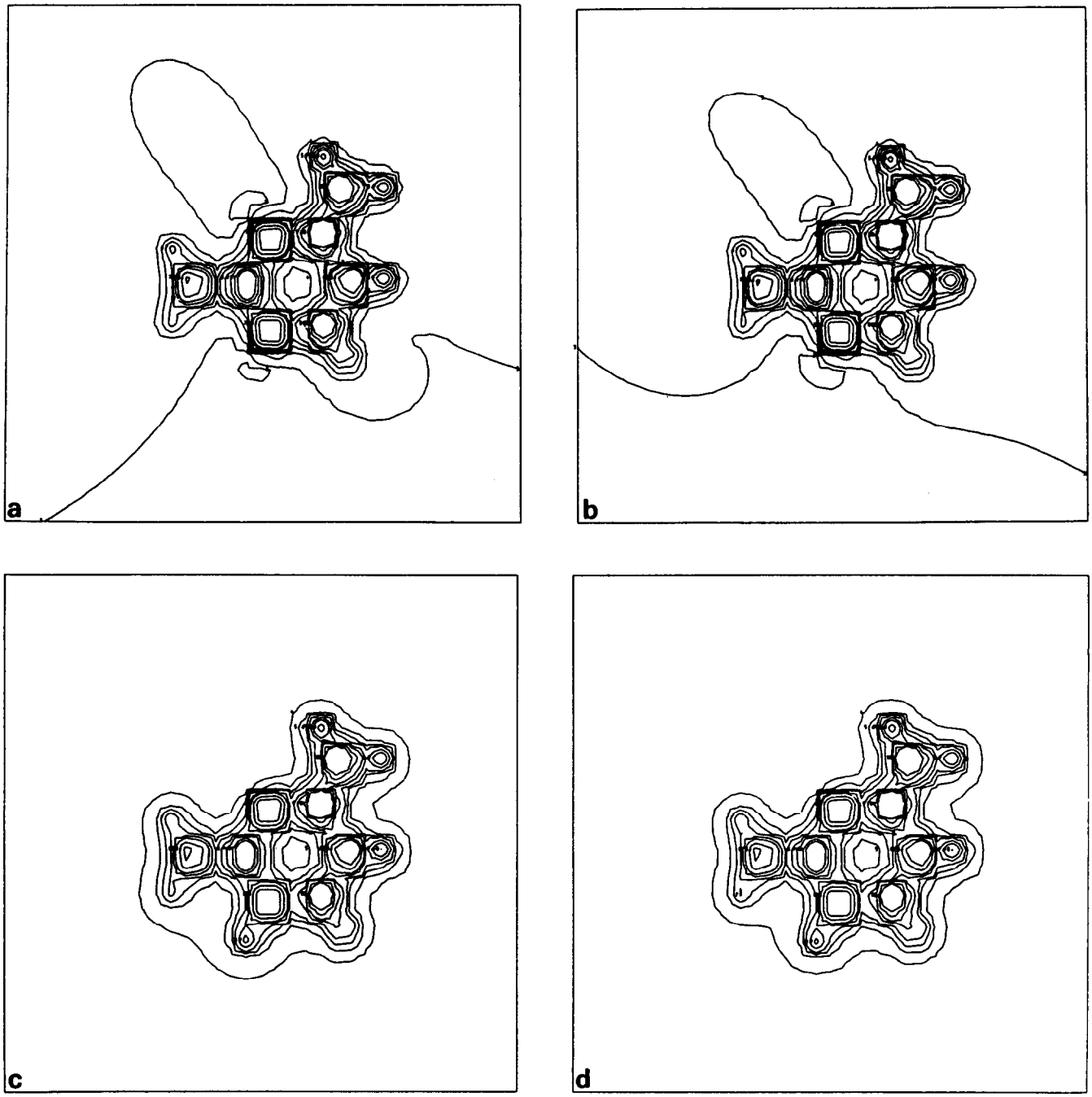


Figure 3. The electrostatic potential of protonated and unprotonated 2,4-diaminopyrimidine with and without partial charges. (a) unprotonated ring with partial charge perturbation, (b) unprotonated ring without partial charge perturbation, (c) protonated ring with binding site partial charges, (d) protonated ring without binding site partial charges

molecule methotrexate. This plot is taken from the ICL Perq computer.

Figure 3 shows the plots of the electrostatic potential for the 2,4-diaminopyrimidine rings; protonated and unprotonated, with and without the partial charge perturbation. They were produced using standard Ghost routines implemented on a VAX computer and contoured at the −0.2, −0.1, 0.0, 0.25, 0.5, 0.75, 1.0, 1.5, 2.0, 3.0, 4.0 atomic unit levels. The values of the charge and the overlap populations calculated from the Mulliken population analyses are shown in Tables 1 and 2 respectively.

DISCUSSION

A recent study by Goodford[10], on the fitting of trimethoprim into the *E. coli* binding site using energy contours that locate the interaction of probe molecules chosen to indicate regions of ionic and hydrophobic interaction, suggests that the amino groups may carry more of the positive charge than had been thought. The values presented in Tables 1 and 2 appear to substantiate this.

The electron density plots (not shown) which were computed for 2,4-diaminopyrimidine show, as is so often the case, no interesting or startling features save for the atomic positions. However, the same cannot be said for the plots of the electrostatic potential.

Figures 3(a) and 3(b) display the electrostatic potential for the unprotonated ring with and without the partial charge perturbation, respectively. From these it can be seen that, by the addition of the binding site partial charges, the lone pair on N3 becomes more diffuse. This is not surprising since the binding site carries an overall doubly positive charge. The electrostatic potential around N1 also varies quite considerably on the addition of the binding site, with the zero contour level being shifted and somewhat more localized. This is the nitrogen to which the proton should be attached, and this change in the position of that potential level probably reflects that this is the site of electrophilic attack.

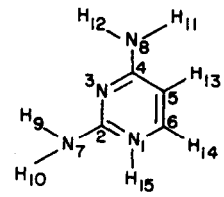
In Figures 3(c) and 3(d) the electrostatic potential of the protonated ring is displayed both with and without the binding site partial charges respectively. These two plots are in contrast to those of Figures 3(a) and 3(b) as here there are very small changes on the addition of the partial charges. The prominent lone pair on N3 has been 'sucked in' on protonation. In addition there are no areas of negative electrostatic potential since the molecule is cationic and the electrostatic energies for the neutral and protonated molecule of +260.4 and −51.2 kJ mol^{-1} reflect this. Similar plots produced in planes 1.5 Å above and below the 2,4-diaminopyrimidine ring show the same salient features as those for the plane of the ring.

It may thus be concluded that in this instance the binding energy is largely of electrostatic origin including in this hydrogen bonding.

ACKNOWLEDGEMENTS

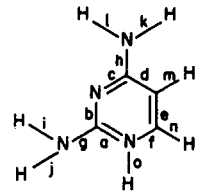
These studies were conducted persuant to a contract with the National Foundation for Cancer Research.

Table 1. Charges for protonated and unprotonated 2,4-diaminopyrimidine, with and without partial charges



Atom charge q(e)	Protonated 2,4-diaminopyrimidine with binding site partial charges	Protonated 2,4-diaminopyrimidine	2,4-diaminopyrimidine with binding site partial charges	2,4-diamino pyrimidine
1	−0.31	−0.30	−0.24	−0.30
2	0.42	0.43	0.32	0.32
3	−0.33	−0.30	−0.36	−0.34
4	0.32	0.34	0.25	0.27
5	−0.12	−0.10	−0.16	−0.14
6	0.09	0.10	0.02	0.02
7	−0.42	−0.42	−0.46	−0.45
8	−0.43	−0.41	−0.46	−0.45
9	0.26	0.28	0.20	0.22
10	0.33	0.25	0.30	0.22
11	0.27	0.28	0.22	0.23
12	0.29	0.29	0.24	0.24
13	0.10	0.13	0.04	0.07
14	0.14	0.15	0.08	0.09
15	0.38	0.28	–	–
Σ	+1	+1	0	0

Table 2. Overlap populations for protonated and unprotonated 2,4-diaminopyrimidine, with and without partial charges



Label overlap pop	Protonated 2,4-diaminopyrimidine with binding site partial charges	Protonated 2,4-diaminopyrimidine	2,4-diaminopyridine with binding site partial charges	2,4-diaminopyrimidine
a	0.41	0.41	0.42	0.42
b	0.42	0.43	0.41	0.42
c	0.44	0.43	0.45	0.45
d	0.44	0.43	0.46	0.46
e	0.57	0.57	0.54	0.55
f	0.39	0.38	0.38	0.38
g	0.43	0.42	0.40	0.40
h	0.45	0.46	0.42	0.43
i	0.36	0.37	0.37	0.37
j	0.36	0.37	0.36	0.37
k	0.37	0.37	0.37	0.37
l	0.36	0.36	0.37	0.37
m	0.41	0.41	0.41	0.41
n	0.41	0.41	0.41	0.41
o	0.34	0.35	–	–
Σ	6.16	6.27	5.77	5.81

REFERENCES

1 **Richards, W G and Cuthbertson, A F** *Chem. Comm.* (1984) p 167
2 **Hayes, D M and Kollman, P A** *J. Am. Chem. Soc.* Vol 98 (1976) p 3335
3 **Blaney, J M, et al.** *J. Am. Chem. Soc.* Vol 104 (1982) p 6424
4 **Schlegel, H B, Poe, M and Hoogsteen, K** *Mol. Pharmacol.* Vol 20 (1981) p 154
5 **Hood, K and Roberts, G C K** *Biochem. J.* Vol 171 (1978) p 357
6 **Cuthbertson, A F and Richards, W G** *J. Mol. Struct.* in press
7 **Hehre, W J, et al.** *Quant. Chem. Progr. Exchange* (1973) p. 236
8 **Peteers, D and Sana, M** *Quant. Chem. Progr. Exchange* (1973) p 360
9 **Bolin, J T, et al.** *J. Biol. Chem.* Vol 257 (1982) p 13650
10 **Goodford, P J** *J. Med. Chem.* in press