Communications to the Editor

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ELECTROSTATIC FORCES IN THE INHIBITION OF DIHYDROFOLATE REDUCTASE BY METHOTREXATE. A FIELD POTENTIAL STUDY

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Methotrexate (MTX) is a high affinity inhibitor of the reaction catalyzed by dihydrofolate reductase (DHFR) from Lactobacillus casei. MTX is a folic acid analogue, being effective in the treatment of neoplastic disease in humans. In order to understand the mechanism of its inhibition from an electrostatic point of view, we calculated electrostatic potentials originating from MTX, NADPH(co-factor)-DHFR complex, NADPH and DHFR, individually. The mutual relationships between MTX and DHFR-NADPH and between NADPH and DHFR were then visualized and compared with the simultaneous geometrical analysis, using a raster computer graphic technique. Complete complementalities of the electrostatic potential and the field electrostatic potential are communicated between MTX and the NADPH-DHFR complex and between NADPH and DHFR.

KEYWORDS —— enzyme; dihydrofolate reductase; methotrexate; NADPH; electrostatic potential; raster graphics; quantum chemistry; molecular orbital

In order to reveal the electrostatic recognition of MTX and NADPH (Fig. 1a) by DHFR, two kinds of electrostatic molecular surfaces on the guest molecules MTX and NADPH were calculated based upon Mulliken net charges from STO-3G ab initio LCAO MO calculations.[1] Moreover they were displayed on colour computer graphics, based on the structure of the ternary complex given by the X-ray analysis (Fig. 1b).[2] The first colour coded potentials on the molecular surfaces of MTX and NADPH as the guest molecules are due to the anisotropic distribution of their respective electron densities. They are called guest potentials on guest molecules (G-on-G potential surfaces). The second field potentials on the molecular surfaces of MTX and NADPH originate from the enzyme-NADPH complex and only the enzyme, respectively, and they are called host potentials on guest molecules (H-on-G potential surfaces).

Figure 2a shows the G-on-G potential surface of MTX on its van der Waals' surface, computed by a graphic program named TERAS.[3] The blue colour code indicates a relatively positive potential and the red code a negative one. The precise potential values are shown to the right of the figure. The pteridine

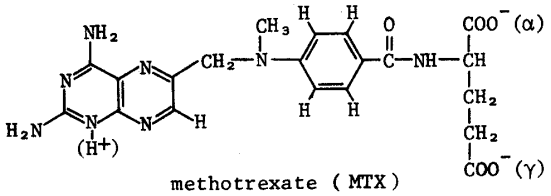
ring, glutamic acid and the p-aminobenzoic acid (PABA) moieties are positive, strongly negative and weakly negative, respectively. Figure 2b shows the H-on-G potential surface of MTX, where the complex of DHFR and NADPH is the host molecule. Here, the 1-hydro and 2-amino groups are strongly negative due to the anionic β-carboxyl group of Asp26, and the 4-amino group is weakly negative due to the carbonyl oxygens of Leu4 and Ala97. The glutamic acid moiety is positive, and the γ-carboxyl group is strongly positive due to the cationic side chains of His28 and Lys51. Evidently, Fig. 2b is the completely reversed potential surface of Fig. 2a, indicating that the electrostatic force is essential in the inhibition of DHFR by MTX.

Figures 3a and 3b show the G-on-G and the H-on-G potential surfaces of NADPH, respectively, on its van der Waals' surfaces. The whole G-on-G potential surface is negative because of the three phosphate groups, and it is strongly negative around the phosphate groups. On the other hand, most of the H-on-G potential surface from DHFR is positive except the adenine site, since there are two cationic arginine residues (Arg43 and Arg44) and two N-termini of α-herices (residues 42-49 and 99-107) near the phosphate groups. In this case also, the H-on-G potential surface is a complete reversal of the G-on-G potential surface.

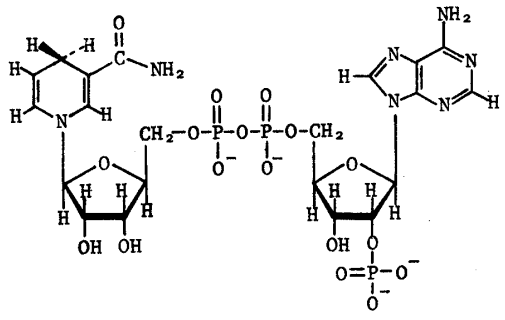
There is a difficulty in estimating correctly the shielding effects produced by the large water dielectric and by the small ions around the molecules. Since the binding sites of MTX and NADPH are not on the surface of DHFR, but in the inner holes, we have tentatively used a small value of $\varepsilon=4$.[4)]

Although it is not rational to compare the electrostatic interaction directly with other short range effects such as steric or hydrophobic interactions, the mechanism of the electrostatic recognition can be understood by visualizing the pairs of the G-on-G and H-on-G potential surfaces. This method may become a powerful tool for a general drug design.

Fig. 1a



methotrexate (MTX)



dihydronicotinamide adenine dinucleotide phosphate (NADPH)

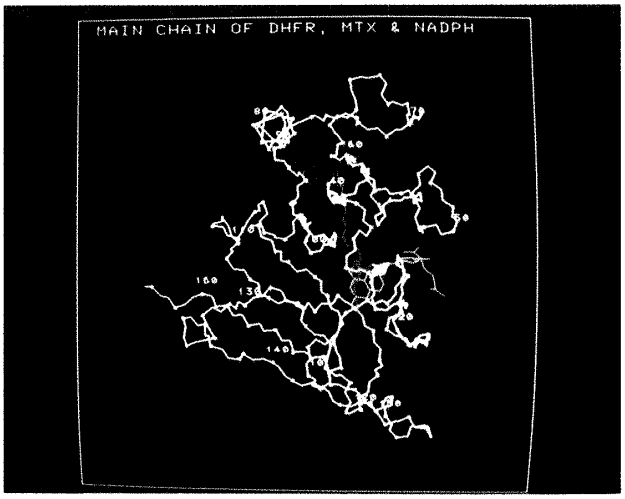


Fig. 1b

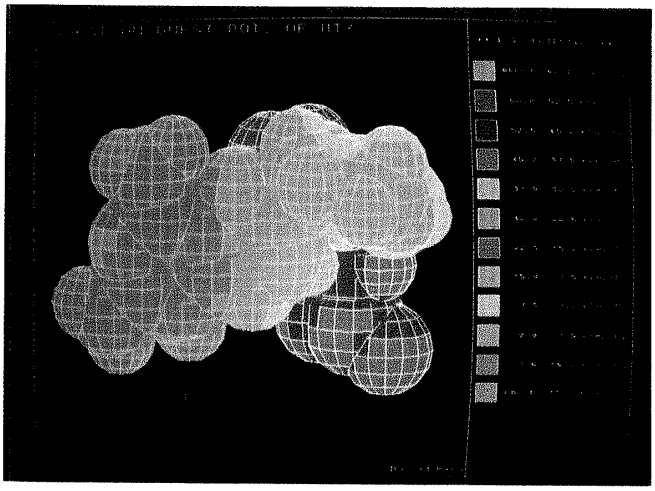


Fig. 2a

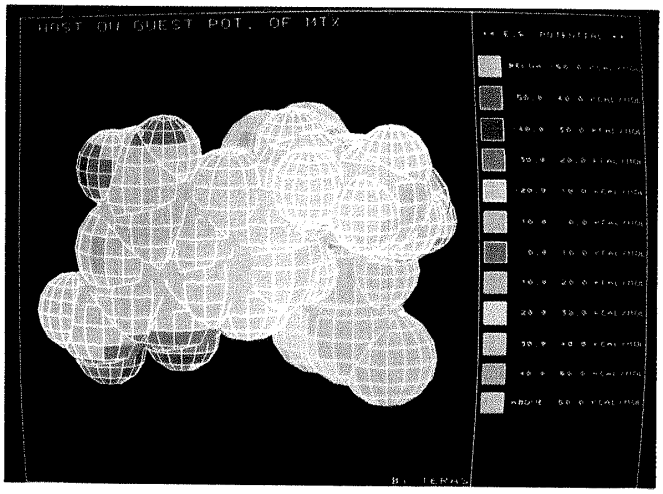


Fig. 2b

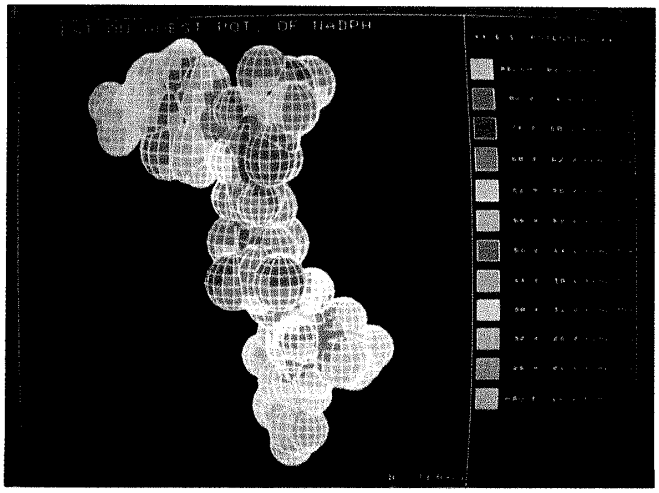


Fig. 3a

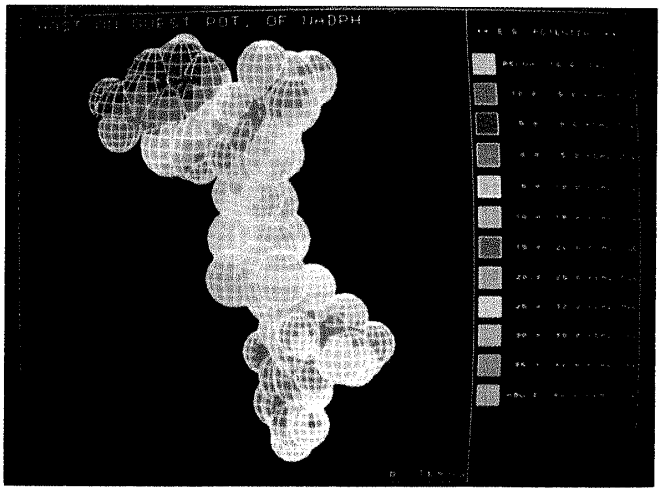


Fig. 3b

FIGURE CAPTIONS

Fig. 1. a: Structure of MTX and NADPH. Since N1 of pteridine ring in MTX appears to be protonated when MTX is bound to DHFR, the 1-hydro form is used.[2] b: Stick model showing the main chain of DHFR, MTX, and NADPH in the ternary complex from Lactobacillus casei.[2] The white, blue and red lines show the main chain, MTX and NADPH, respectively, and asterisks correspond to α-carbon atoms of amino acid residues.

Fig. 2. The raster graphic illustration showing the colour coded electrostatic potential on the van der Waals' surface of MTX. a: G-on-G potential surface, where MTX is the guest molecule. b: H-on-G potential surface, where the complex of DHFR and NADPH is the host molecule.

Fig. 3. The raster graphic illustration showing the colour coded electrostatic potential on the van der Waals' surface of NADPH. a: G-on-G potential surface, where NADPH is the guest molecule. b: H-on-G potential surface, where DHFR is the host molecule.

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