Binding of Methotrexate to Dihydrofolate Reductase by Quantum Chemical Calculation

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Quantum mechanical calculations give a good prediction of the binding of methotrexate to dihydrofolate reductase.

In principle (as theoreticians always say) it should be possible to start with a refined protein crystal structure and to compute the binding energy of known or putative ligands by pure quantum mechanical *ab initio* molecular orbital methods. The seductive brute-force approach is precluded only because it would demand unrealistic amounts of computer time. An approximation to this procedure[1,2] becomes viable if one replaces the atoms of the enzyme binding site with partial point charges which allow for the screening of the positive atomic nuclei by electron densities. This is achieved by altering a standard *ab initio* molecular orbital package so that partial point charges may be introduced as dummy atoms which possess a non-integral nuclear charge but no electrons. The result is many extra electron–nuclear integrals which are readily computed, without adding more electron–electron interaction integrals. If the technique can reproduce experimental binding energies then it opens the way to test the effect of chemical modification prior to synthesis and hence predict novel enzyme inhibitors for such systems as dihydrofolate reductase (DHFR) whose blockade should lead to anti-cancer therapeutic agents.

The system reported here is the methotrexate complex with *Lactobacillus casei* DHFR. The co-ordinates of the enzyme–inhibitor complex were obtained from the Brookhaven Database[3] and the residues in the binding site important to the binding of methotrexate to DHFR identified. This comprises of the seventeen residues: Leu 4, Trp 5, Ala 6, Leu 19, Trp 21, Asp 26, Leu 27, His 28, Phe 30, Arg 31, Ser 48, Phe 49, Pro 50, Leu 54, Arg 57, Ala 97, and Thr 116. The atoms in the binding site were replaced by their respective appropriate partial point charges.[4]

The calculations were performed with a modified version of the Gaussian '70 program[5] implemented on an ICL 2988 computer. By subtracting the calculated total energy of methotrexate from the energy of the methotrexate plus the partial charges an idea of the binding energy of the inhibitor to the enzyme can be obtained.

Because of the size of methotrexate (**1**) the calculation is performed on six smaller fragments, A → F; the binding energies of which can be found in Figure 1. E_T(i) refers to the total energy of fragment i and E_T(i DHFR) to the total energy of the fragment plus the enzyme partial charges. The discontinuities give rise to a few extraneous atoms (X), which will add to the energy of the system and so their energy has to be removed from the calculation. This is achieved in a similar manner to that for the enzyme-inhibitor complex.

The binding energy is then given by equation (1).

$$\text{Binding energy} = \sum_{i=A}^{F} [E_T(\text{i DHFR}) - E_T(\text{i})] - [E_T(\text{X DHFR}) - E_T(\text{X})] \quad (1)$$

The computing binding energy is −62.69 kJ mol^{-1}. This energy is ΔU of binding. Presumably since no volume changes are involved this is equal to the enthalpy ΔH. Experimentally[6] the free energy of binding, ΔG, is −48.49 kJ mol^{-1}. Although

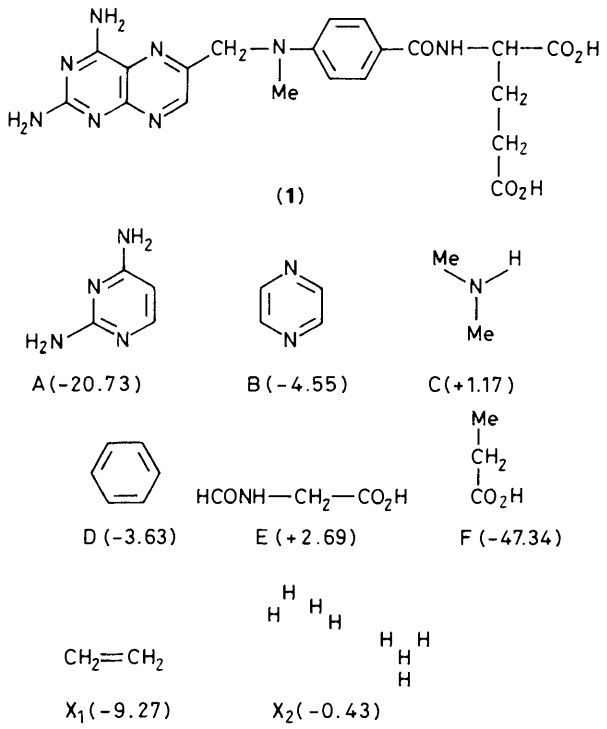

Figure 1. Methotrexate (**1**), and the fragments (A—F) and X_1 and X_2 with their computed contributions to binding (kJ mol^{-1}, with negative values indicating attraction).

on binding the ligand will lose motional entropy it is possible to estimate the more important entropic changes due to solvent. Calorimetric measurements by Subramanian and Kaufman[7] have shown that ΔS for the *Escherichia coli* enzyme is 22.6 J K^{-1} mol^{-1}. Assuming similar values are appropriate for the *L. casei* enzyme, $T\Delta S$ at 298 K is −6.7 kJ mol^{-1} which makes our calculation of ΔH agree with experiment to within 10 kJ mol^{-1}. It does appear that the computed binding energy is a good enough estimate to be used in comparative studies on modified ligands. Care will have to be exercised in allowing for changes in the atomic positions of the enzyme atoms on binding new ligands and these are probably best estimated using computer graphic techniques.

The relatively simple procedure does open the way to theoretical screening.

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