Pterin 1*H*–3*H* Tautomerism and its Possible Relevance to the Binding of Folate to Dihydrofolate Reductase

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Ab initio and free energy perturbation calculations show that the 1*H*:3*H* tautomer ratio is 1 : 6 for pterin in aqueous solution, implying that the 1*H* tautomer of folate may be responsible for non-productive binding to dihydrofolate reductase.

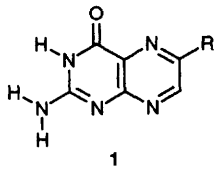
Dihydrofolate reductase (DHFR) catalyses the reduction not only of dihydrofolate, but also of folate, **1** with an efficiency that varies between species. It is the target for several important drugs including the anticancer agent methotrexate **2**. Crystallographic studies on bacterial[1] and mammalian[2] DHFR have led to the conclusion that **1** binds to DHFR with its pteridine ring rotated approximately 180° about an axis through the C(2)–NH_2 bond from the orientation in bound **2**. However, NMR studies[3] on **1** with DHFR from *Lactobacillus casei* and $NADP^+$ at alkaline pH have shown binding of **1** in two alternative modes with a ratio of 1 to 1.67: nonproductively (IIa), with its pteridine ring orientation resembling that of **2**, as well as productively (IIb), with its pteridine ring oriented as in the crystal structures. We propose that tautomerism in the pterin system of **1** may influence the mode of binding.

Theoretical and spectroscopic studies of pterin [2-amino-4(1*H*)-pteridinone, **3a, b**] agree that the oxo form, conventionally written as the 3*H* tautomer **3a**, is more stable than the enol form. Less attention has been paid to the 1*H* tautomer **3b** despite the fact that in the corresponding single-ring system isocytosine [2-amino-4(1*H*)-pyrimidinone] an equimolar mixture of 1*H* and 3*H* tautomers is observed both in the crystal structure[4] and in aqueous media.[5,6] Indeed, crystalline 6-methylisocytosine exhibits the 1*H* tautomer exclusively.[7] According to *ab initio* calculations[8] the isolated pterin molecule **3b** is less stable than **3a** by 23 kJ mol^{-1} in the STO-3G basis and 28 kJ mol^{-1} in 3-21G. This difference is consistent with the greater separation between the positively charged NH hydrogen atom and the negative carbonyl oxygen atom in the (vinylogous) amide of **3b** than **3a**, but it may be mitigated by solvation in the more biologically relevant aqueous phase. Therefore, we have continued the *ab initio* studies to larger basis sets and considered the solvation effects.

Full restricted Hartree–Fock geometry optimisations[9] were carried out on both the 1*H* and 3*H* tautomers with STO-3G, 3-21G and 6-31G* basis sets; finally, single point MP2/6-31G* calculations were performed to find the 1*H*–3*H* gas-phase energy difference. The difference in solvation free energies between the tautomers was calculated using free energy perturbation theory within Monte Carlo simulations.[10] Essentially, these calculations involve mutating a proton into a massless dummy atom in one position and growing a proton from a dummy in the other, effectively giving a tautomeric change. 3*H* Pterin was mutated to 1*H* pterin in a periodic box of 500 TIP4P water molecules.[11] Solute parameters were taken from the OPLS nucleic acid force field[12] with the optimised 3-21G solute geometry. The perturbation was performed over 21 windows, each comprising 3×10^5 configurations of NVT equilibration, 4×10^6 configurations of NPT equilibration and 5×10^6 configurations of NPT data collection. Only intermolecular interactions were evaluated in the simulations and a non-bonded cut-off of 7.5 Å was used for all intermolecular interactions.

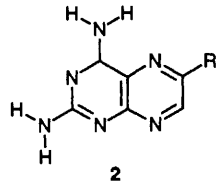
Forward and backward perturbations showed good agreement with hysteresis only 1.09 kJ mol^{-1}. The free energy change of −20.46 kJ mol^{-1} on mutating solvated **3a** to solvated **3b** nearly cancels the gas-phase energy advantage of **3a** (Table 1). Based on the MP2/631G* calculations, $\Delta G°$ for the process **3a** → **3b** is +4.52 kJ mol^{-1}, yielding an equilibrium constant of 0.162 at 298 K.

The K_{eq} calculated for pterin in aqueous solution suggests that one folate molecule in seven could exist as the 1*H* tautomer, presenting to DHFR the same pattern of hydrogen-bond donors and acceptors in its ring positions 1–3 as



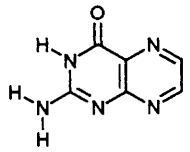
1

Folate, R = -$CH_2NHC_6H_4CO$-glutamate,
Folic acid, R = -$CH_2NHC_6H_4CO$-glutamic acid

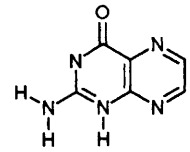


2

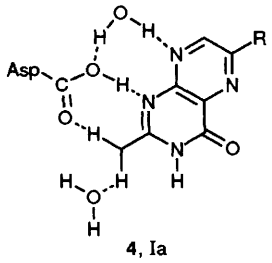
Methotrexate, R = -$CH_2MeC_6H_4CO$-glutamate



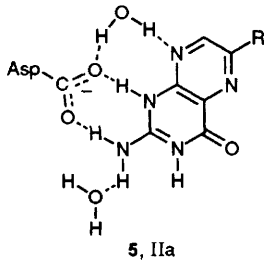
3a, 3*H* tautomer



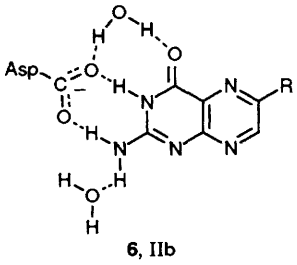
3b, 1*H* tautomer



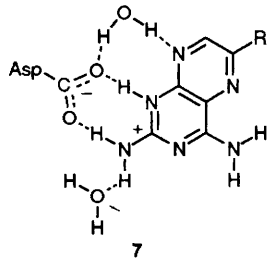
4, Ia



5, IIa



6, IIb



7

Methotrexate–DHFR binding conformation

Table 1 Calculated *ab initio* energies and 3*H*/1*H* tautomer solution phase equilibrium constants.

Basis set	1*H*–3*H* ΔE_{gas}/ kJ mol^{-1}	3*H*/1*H* K_{eq}
RHF/STO-3G	16.52	4.909
RHF/3-21G	22.34	0.469
RHF/6-31G*	25.78	0.117
MP2/6-31G*	24.98	0.162

methotrexate. In the *L. casei* DHFR-folate-NADP$^+$ complex binding of this tautomer in orientation IIa should be facile, while the 3*H* tautomer should bind more readily in IIb. Tautomer abundance and relative binding affinity could jointly yield the observed 1:1.67 ratio. While IIa superimposes the carboxy oxygen atom O(4) on an amino group of methotrexate that points at backbone carbonyl oxygen atoms, in IIb this same site is occupied by pyrazine N(8). Any repulsive interactions will be roughly equal, O(4) being <0.07e more negative according to molecular electrostatic potential fitted atom-centred charges calculated from 6-31G* wave functions and just 0.01e more negative in the OPLS nucleic acid force field.[12] The conformational equilibrium shows pH dependence[3] probably due to the active-site aspartate Asp-26. At pH <5.5 there is only a single conformation I which is non-productive and resembles IIa. A consistent hydrogen bonding scheme, **4**–**7** would result if at alkaline pH both tautomers were to bind to an anionic Asp-26, while at acidic pH only the more abundant 3*H* tautomer might bind in the more favourable orientation to the neutral Asp-26. The environment of the Asp carboxy group could enhance or abolish the effect: no alternative conformations for folate have been found in crystallographic[1] and NMR[13] studies of complexes of *Escherichia coli* DHFR with folate, but the crystal structure of human DHFR with folate exhibits disorder in the vicinity of the N5 of folate.[2] It is tempting to ascribe this disorder to partial occupancy by the 1*H* tautomer.

This work was conducted in part pursuant to a contract with the National Foundation for Cancer Research and supported by the Association for International Cancer Research. D. R. L. is supported by an SERC CASE studentship with Eli Lilly Ltd.

Received, 29th April 1993; Com. 3/02472E

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