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Ab Initio Calculations on Keto/Enol Tautomers of Pterins. On the Significance of the Enol Tautomer of Pterin Ring in the Enzymatic Reduction of Dihydrofolate by DHFR

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Ab initio and semi-empirical calculations were carried out on keto/enol tautomers of 6-methyl-7,8-dihydropterin, a model compound for the natural substrate of dihydrofolate reductase. The most likely conformer of pterin ring (inner enol **3**) during the hydride transfer from NADPH was computationally chracterized.

During the last three decades, the metabolic importance and versatility of dihydrofolate reductase (DHFR) have inspired extensive researches on the relation between the enzyme structure and the reaction kinetics.[1] DHFR catalyzes the hydride transfer from NADPH to the imino carbon atom (C6) of 7,8-dihydrofolate, yielding the imino-double-bond (C6=N5) reduced product, 5,6,7,8-tetrahydrofolate.

The key amino acid residue for the catalytic efficiency has proven to be Asp-27 (Escherichia coli numbering) by means of site-directed mutagenesis.[2] Asp-27 is strictly conserved among all DHFRs so far isolated. The mutation from Asp to Asn at the position 27 has resulted in entire loss of enzymatic protonation activity. In the crystallographic structure of the E. coli DHFR-methotrexate(MTX) binary complex,[3] Asp-27 is located at the dihydrofolate binding site, forming a pair of hydrogen bonds with the pterin ring portion of MTX. According to the proposed binding of the actual substrate, however, the Asp-27 group is more than 5 Å away from the nitrogen atom (N5) of the bound dihydrofolate.[2,3] It is thus hardly conceivable that proton is directly transferred from Asp-27 to the dihydrofolate imino nitrogen (N5). Consequently, there ought to exist a chemical mechanism mediating a proton shuffling between Asp-27 carboxyl group and the imino nitrogen atom. Recently, the significance of enol form of pterin ring has been pointed out: the enolic proton is presumably transferred to the imino nitrogen atom in the enzymatic process due to the action of Asp-27.[4] According to this hypothesis, we have carried out ab initio and semi-empirical calculations on 6-methyl-7,8-dihydropterin as a model substrate in order to computationally characterize keto and enol forms of the pterin ring.

Three isomers of 6-methyl-7,8-dihydropterin, keto form **1**, outer enol form **2**, and inner enol form **3**, were fully optimized with the STO-3G minimal basis set without any constraint.[5] As shown in Fig. 1, the pyrimidine ring portion remains almost planar in each isomer, whereas the methylene carbon in the

dihydropyrazine portion deviates from the plane of the pterin ring. The N3-C4 bond distance of 1.45 Å in 1 decreases upon enolization to 2 and 3 (to 1.36 Å). This structural change is not translated into the adjacent dihydropyrazine ring containing the imino double bond. The bond shortening at N3-C4 is accommodated inside the pyrimidine ring by readjusting a few bonds, such as N1-C2 and C4-C10 (see Table 1).

Full geometry optimizations with STO-3G basis set on the corresponding three isomers (keto, outer enol, and inner enol) of 7,8-dihydropterin were also carried out. The effect of 6-methyl group on the geometry is hardly recognized: the optimized structure of each isomer shows almost the same conformation of pterin ring portion as in the corresponding 6-methyl derivative. The absence of 6-methyl group, however, produces remarkable changes on the electron populations estimated on the imino carbon (C6) and nitrogen (N5) atoms. More specifically, the atomic charges on the imino double bond of inner enol 3 are calculated to be +0.138 and -0.264, respectively, for C6 and N5, whereas the corresponding charges of 7,8-dihydropterin are +0.062 and -0.248. The nucleophlic attack at C6 is presumably accelerated by methyl (actually anilinomethyl group in dihydrofolate) substitution at the position 6 owing to the electronic effect.

The relative energies of keto and enol forms of pterin ring were estimated from several computational levels.[6] Not surprisingly, the stability difference between keto and enol forms estimated from the STO-3G total energies conflicts with the experimental results.[7] The STO-3G calculations indicate that outer and inner enol forms 2 and 3 are more stable than keto form 1 by 13.6 and 14.0 kcal/mol, respectively. The STO-3G minimal basis sets may be adequate to determine relative changes in the structure. However, relative stabilities predicted from minimal basis calculations are not always satisfactory,[8] as experienced by Gready in his *ab initio* studies on pterin ring systems.[9] Slightly different estimates are given with semi-empirical calculations. AM1 Hamiltonian indicates that outer and inner enol forms 2 and 3 are 0.5 and 0.1 kcal/mol more stable than keto form 1 on the STO-3G optimized geometry. On the AM1 optimized geometries, however, enol forms 2 and 3 are less stable than keto form 1 by 0.6 and 0.8 kcal/mol, respectively. Single-point calculations with split-valence basis sets on the STO-3G optimized geometries provide the following estimates. The outer enol form 2 is indicated to be less stable than keto form 1 by 1.4 and 1.0 kcal/mol, respectively, with 3-21G and 6-31G basis sets. The inner enol form 3, on the other hand, is indicated to be more stable than 1 by 0.4 and 0.3 kcal/mol with these basis sets. Thus, semi-empirical and *ab initio* calculations both indicate that stability difference between three isomers of pterin ring 1, 2, and 3 are minimal. More importantly, the inner enol form 3 is found to be the most stable isomer with *ab initio* calculations, regardless of the basis sets utilized.

According to the theoretical relative energy estimates, all of three isomers of pterin ring, keto form 1 and enol forms 2 and 3, are conceivably involved in DHFR enzymatic reduction process. It is not unreasonable to presume rather rapid enzymatic tautomerization between these three isomers. The significance of inner enol form 3 in the enzymatic process is supported by probable weak interaction between the imino nitrogen atom (N5) and the enolic proton. The interatomic

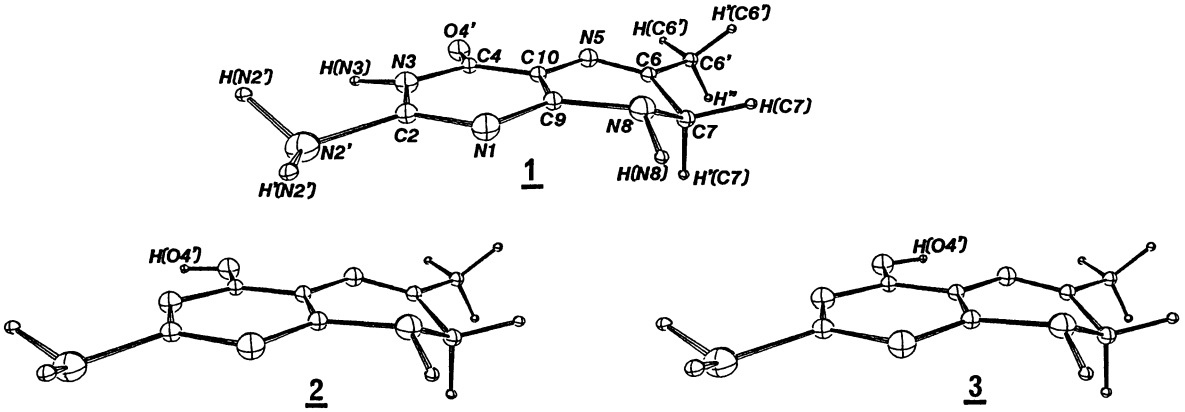

Fig. 1. The STO-3G optimized structures of keto 1, outer enol 2, and inner enol 3 forms of 6-methyl-7,8-dihydropterin.

Table 1. The STO-3G structural parameters

Bond Lengths	1	2	3
N1 - C2	1.309	1.359	1.364
C2 - N3	1.388	1.359	1.360
N3 - C4	1.450	1.356	1.356
C4 - C10	1.484	1.399	1.399
C10 - N5	1.448	1.448	1.444
N5 - C6	1.281	1.281	1.281
C6 - C7	1.542	1.542	1.544
C7 - N8	1.476	1.476	1.477
N8 - C9	1.418	1.414	1.415
C9 - C10	1.351	1.399	1.395
C2 - N2'	1.429	1.423	1.421
C4 - O4'	1.220	1.376	1.375
C6 - C6'	1.526	1.526	1.526
H - N2'	1.028	1.026	1.025
H - N2'	1.027	1.026	1.025
H - N3	1.021	-	-
H - O4'	-	0.990	0.991
H - C6'	1.085	1.085	1.085
H - C6'	1.088	1.088	1.088
H - C6'	1.088	1.088	1.088
H - C7	1.092	1.092	1.092
H - C7	1.099	1.098	1.098
H - N8	1.026	1.025	1.026

Bond Angles	1	2	3
C2 - N1 -C9	113.4	113.5	113.3
N1 - C2 -N3	125.6	128.7	129.4
N1 - C2 -N2'	119.0	115.6	115.3
N2' - C2 -N3	115.4	115.7	115.3
C2 - N3 -C4	123.4	114.3	113.8
C2 - N3 -H(N3)	118.5	-	-
H(N3)- N3 -C4	117.9	-	-
N3 - C4 -C10	110.7	123.6	123.1
N3 - C4 -O4'	120.0	116.3	116.0
O4' - C4 -C10	129.3	120.1	120.9
C10 - N5 -C6	116.6	116.0	115.9
N5 - C6 -C7	124.3	124.8	124.4
N5 - C6 -C6'	119.5	119.2	119.6
C6' - C6 -C7	116.2	116.0	116.0
C6 - C7 -N8	109.3	110.0	110.6
C6 - C7 -H(C7)	110.0	109.7	109.5
C6 - C7 -H'(C7)	109.3	109.1	108.6
H(C7)- C7 -N8	108.7	108.6	108.6
H'(C7)- C7 -N8	112.2	112.1	112.1
H(C7)- C7 -H'(C7)	107.3	107.3	107.3
C7 - N8 -C9	115.5	115.7	115.7
C7 - N8 -H(N8)	114.2	114.4	114.2
H(N8)- N8 -C9	111.8	112.3	112.5
N1 - C9 -N8	113.8	117.4	118.3
N1 - C9 -C10	126.6	124.4	123.7
N8 - C9 -C10	119.5	118.2	117.9
C4 - C10 -N5	116.9	121.4	119.3
C4 - C10 -C9	120.4	115.5	116.8
C9 - C10 -N5	122.6	123.0	123.9
C2 - N2 -H(N2')	111.9	111.7	111.7
C2 - N2 -H'(N2')	109.4	111.6	111.9
H(N2')- N2 -H'(N2')	109.7	111.5	111.8
C4 - O4' -H(O4')	-	103.7	103.7
C6 - C6' -H(C6')	109.8	109.8	110.0
C6 - C6' -H'(C6')	110.5	110.4	110.4
C6 - C6' -H''(C6')	110.5	110.4	110.4
H(C6')- C6' -H'(C6')	109.0	109.1	109.0
H'(C6')-C6' -H''(C6')	107.9	108.0	108.0
H(C6')- C6' -H''(C6')	109.1	109.1	109.1

Torsional Angles	1	2	3
N1 -C2 -N3 -C4	+1.7	+0.8	+1.5
C2 -N3 -C4 -C10	-1.1	+0.1	+0.3
C2 -N3 -C4 -O4'	+178.7	+179.9	-179.8
N3 -C4 -C10-C9	+0.6	-0.8	-1.6
N3 -C4 -C10-N5	-177.0	-178.3	-179.0
C4 -C10-C9 -N1	-0.4	+0.6	+1.5
C4 -C10-C9 -N8	-176.2	-175.6	-174.5
C4 -C10-N5 -C6	-169.5	-167.0	-171.3
C4 -N3 -C2 -N2'	-175.6	-176.3	-176.0
N5 -C6 -C7 -N8	-27.7	-27.5	-26.8
C6 -C7 -N8 -C9	+39.6	+40.2	+39.1
C7 -N8 -C9 -N1	+154.3	+153.6	+154.4
C7 -N8 -C9 -C10	-29.4	-29.9	-29.4
N8 -C9 -N1 -C2	+176.7	+176.4	+176.0
N8 -C9 -C10-C4	-176.2	-175.6	-174.5
N8 -C9 -C10-N5	+1.3	+1.9	+2.8
N8 -C7 -C6 -C6'	+154.3	+154.4	+155.6
C9 -N1 -C2 -N2'	+175.8	+176.2	+175.9
C10-N5 -C6 -C6'	-180.0	+179.8	+179.7
N1 -C2 -N2'-H(N2')	+137.2	+153.9	+155.3
N1 -C2 -N2'-H'(N2')	+15.4	+28.3	+29.2
N1 -C2 -N3 -H(N3)	+176.0	-	-
N3 -C4 -O4'-H(O4')	-	-0.2	+178.0
N5 -C6 -C6'-H(C6')	-2.3	-2.4	-2.4
N5 -C6 -C6'-H'(C6')	+118.0	+117.9	+118.0
N5 -C6 -C6'-H''(C6')	-122.6	-122.8	-122.8
N5 -C6 -C7 -H(C7)	-146.9	-146.9	-146.4
N5 -C6 -C7 -H'(C7)	+95.5	+95.9	+96.7
C6 -C7 -N8 -H(N8)	+171.4	+173.0	+172.0

distance between these two atoms of inner enol form 3 is calculated to be 2.20 Å in the STO-3G optimized geometry. Both ab initio and semi-empirical calculations indicate that, although overall electron population in enol form 3 is very similar to that of enol form 2, the electron population on the imino double bond (C6=N5) and the enolic proton changes upon rotation of the enolic hydroxyl group (2→3), implicating a significant interaction between the enolic hydrogen and N5 in 3, i.e., based on the minimal and split-valence basis set calculations, the charge separation in imino double bond (C6=N5) and positive charge on the enolic proton increase in inner enol form 3.[10] The stability difference between 2 and 3 in the ab initio calculations probably originates from this hydrogen-bonding-like interaction. In the actual enzymatic process, this interaction appears to activate the C6=N5 double bond in 3 for the nucleophlic hydride attack and the enolic proton ultimately migrates to N5 upon completion of the reduction.

In conclusion, Asp-27 is presumed to donate its carboxyl proton to the pyrimidine ring portion of dihydrofolate, catalyzing the enolization to 3. Then, the hydride transfer from NADPH to the imino carbon atom (C6) of dihydrofolate is most likely to occur on inner enol form 3, concomitant with migration of the enolic proton to the imino nitrogen atom (N5).

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10) The following atomic charge estimates are obtained with 6-31G single-point calculations for keto/enol isomers 1, 2, and 3: +0.263, +0.263, +0.287 for C6 and -0.472, -0.464, -0.562 for N5. The atomic charge on the enolic proton is calculated to be +0.434 and +0.454, respectively, for 2and 3.

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