Vol. 161, No. 1, 1989 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
May 30, 1989 Pages 64-68

COMPUTATIONAL STUDIES ON PTERINS AND SPECULATIONS ON THE MECHANISM OF ACTION OF DIHYDROFOLATE REDUCTASE

Tadafumi Uchimaru[=], Seiji Tsuzuki[=], Kazutoshi Tanabe[=], Stephen J. Benkovic[+], Kensuke Furukawa[#], and Kazunari Taira[#*]

[=]National Chemical Laboratory for Industry &
[#]Fermentation Research Institute,
Agency of Industrial Science and Technology, MITI
Tsukuba Science City 305, Japan

[+]Department of Chemistry, The Pennsylvania State University
University Park, Pennsylvania 16802

Received March 23, 1989

Summary: The significance of the enol form of the pterin ring in enzymatic reduction of dihydrofolate by DHFR is discussed on the basis of the results of *ab initio* calculations carried out on the keto/enol tautomers of 6-methyl-7,8-dihydropterin as the model compound for the natural substrate, dihydrofolate.

Dihydrofolate reductase (5,6,7,8-tetrahydrofolate: $NADP^+$ oxidoreductase, EC 1.5.1.3; DHFR) catalyzes the reduction of 7,8-dihydrofolate to 5,6,7,8-tetrahydrofolate with NADPH as a cofactor. DHFR is necessary for maintaining intracellular pools of tetrahydrofolate and its derivatives which are essential cofactors in many important metabolic reactions, such as the biosynthesis of thymidylate, purine nucleotides and various one-carbon unit transfer reactions. Consequently, DHFR is a target site of the action of the anti-folate drugs, including methotrexate (MTX), trimethoprim and pyrimethamine. The metabolic importance of DHFR has inspired extensive researches, both on its structure and kinetics, over the past three decades (1). DHFR is one of a few proteins whose X-ray crystal structure has been solved to a resolution better than 2 A (2,3).

*To whom correspondence should be addressed.

Abbreviations: DHFR, dihydrofolate reductase; MTX, methotrexate.

0006-291X/89 $1.50

The key amino acid residue at the active site of DHFR is Asp-27 (Escherichia coli numbering), which is strictly conserved among all DHFRs so far isolated. The functional role of Asp-27 as a proton donor has been resolved by site-directed mutagenesis of E. coli DHFR (4). According to the crystallographic structures of DHFR-MTX complexes, Asp-27 is located at the dihydrofolate binding site and interacts with the pterin ring of MTX, forming a pair of hydrogen bonds with N1 and 2-amino group (Fig. 1b) (2,3). The pteridine ring of MTX, however, faces in the opposite direction as compared to the orientation of the pterin ring of dihydrofolate in the binding site (5). Furthermore, DHFR-bound MTX is found to be protonated at N1, whereas bound dihydrofolate is not protonated (6,7).

According to the probable hydrogen-bonding interactions between dihydrofolate and DHFR, Asp-27 is hydrogen-bonded to the 2-amino group and to N3 of the substrate but it is more than 5 Å away from the imino N5 (2-4). Thus, the direct proton transfer from Asp-27 to imino N5 of dihydrofolate is not feasible. Nevertheless, there ought to exist a chemical mechanism with which the proton channels from Asp-27 to imino N5, since Asp-27 is proven to be the sole proton source (4). It has been proposed that the proton transfer could proceed via enol form of the pterin ring. According to this hypothesis, we have theoretically examined keto/enol tautomerization mechanism utilizing 6-methyl-7,8-dihydropterin 1 as a model substrate.

METHODS OF CALCULATIONS

All of the calculations were performed with the GAUSSIAN 86 program (8) on an FACOM M780/MSP computer. The geometries were fully optimized at the Hartree-Fock level with analytical gradients, using the STO-3G minimal basis set. Neither symmetry nor any constraint was enforced in the geometry optimization. Single-point calculations were carried out on the optimized geometries using the 3-21G and the 6-31G split valence basis set.

RESULTS AND DISCUSSION

The total energy differences are given in Table 1 for 6-methyl-7,8-dihydropterin 1 and its enol forms 2 and 3. The inadequacy of the STO-3G basis set for prediction of relative energies between keto/enol tautomers has been documented (9) and similar basis set discrepancies for pterin N-H/O-H tautomer pairs has been found by Gready, who carried out pioneering theoretical investigations on the pteridine systems (10-12).

Table 1. Relative energies and atomic charges of the tautomers of 6-methyl-7,8-dihydropterin

computational level	keto form 1[a)]	enol form 2	enol form 3
relative energies[b)]			
RHF/STO-3G	0.00	-13.55	-13.99
RHF/3-21G//STO-3G	0.00	1.42	- 0.41
RHF/6-31G//STO-3G	0.00	1.02	- 0.25
atomic charges			
RHF/3-21G//STO-3G			
N5	-0.602	-0.608	-0.680
C6	+0.315	+0.311	+0.335
RHF/6-31G//STO-3G			
N5	-0.472	-0.464	-0.562
C6	+0.263	+0.263	+0.287

a) The STO-3G, 3-21G and 6-31G total energies of 1 are -609.60503, -613.96553 and -617.13667 au, respectively.
b) Relative energies with respect to 1 (kcal/mol).

Thus, the total energies calculated with the STO-3G basis set, indicating that enol form of the 6-methyl-7,8-dihydropterin 2 is more stable than keto form 1 by 13.6 kcal/mol, clearly conflict with the experimental results. The split valence basis sets are expected to give more reliable relative energies between keto/enol tautomers (12). As shown in Table 1, single-point calculations on the STO-3G optimized geometries using split valence basis sets, 3-21G and 6-31G, reveal that keto form pterin 1 is more stable than its enol form 2 by 1.4 and 1.0 kcal/mol, respectively. However, more importantly, the other enol form 3 is found to be at least as stable as keto form 1 at all levels of basis sets utilized in the present calculations. Enol form 3 differs from 2 only by the orientation of the enolic hydrogen, i.e. the dihedral angle N3-C4-O4'-H. Recently, Ewig et al. has reported that even STO-3G minimal basis set calculations give a reliable indication with respect to the torsional angles and the relative energies of the conformers of various phosphorous acids and esters (13). Thus, it is not surprizing to find in Table 1 that enol form 3 is calculated to be more stable than enol form 2, regardless of the basis sets utilized, reflecting reliable relative stability between enol forms 2 and 3.

The most likely cause for stability difference between 2 and 3 originates from the hydrogen bonding interaction between imino N5 and enolic hydrogen, which is possible only in the conformer 3. The electron density on C6 estimated by the calculations using minimal and split valence basis sets also supports weak

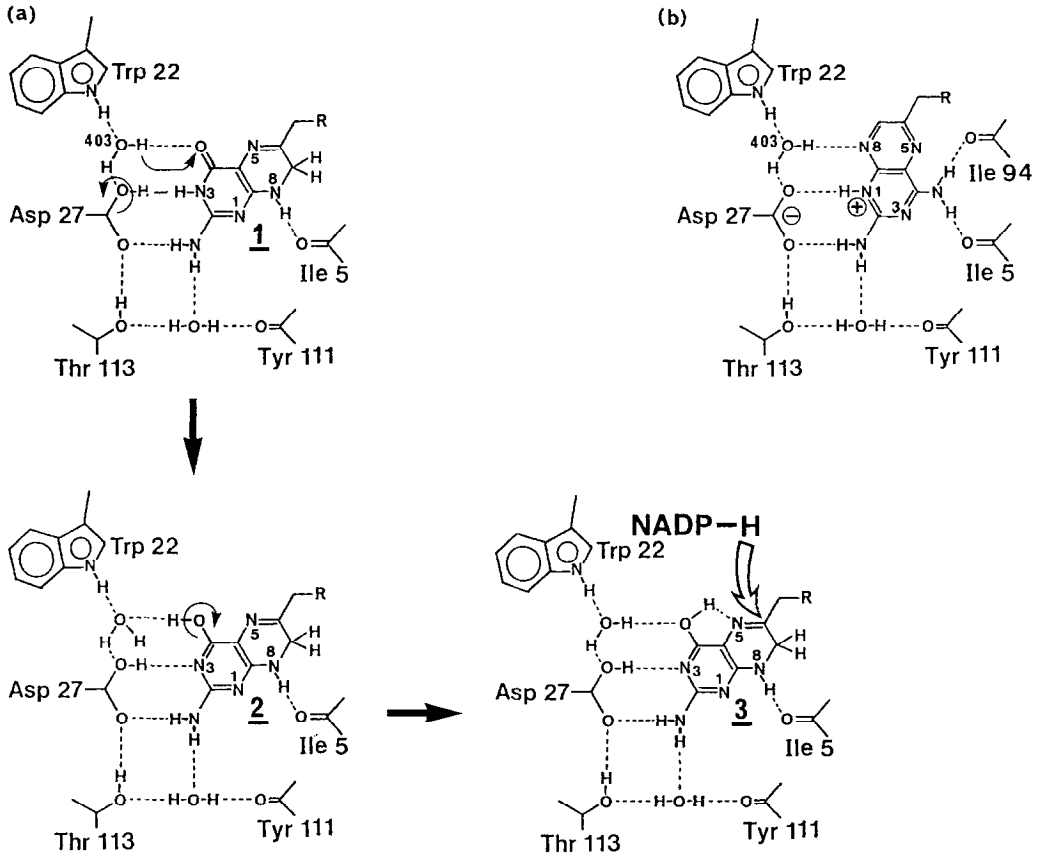

Figure 1. (a) Proposed chemical mechanism (14) of the proton transfer from Asp-27 of dihydrofolate reductase (DHFR) to the N5 of the substrate, dihydrofolate (R=H in the ab initio calculations). (b) Hydrogen bonding interaction between DHFR and the pterin portion of methotrexate (MTX).

hydrogen bonding interaction in enol form 3. Compared with either keto form 1 or enol form 2, the positive charge on C6 increases in 3 (Table 1). Therefore, imino N5 is considered to be partially protonated in 3. The hydride transfer from the cofactor, NADPH, to imino carbon C6 is probably facilitated by the partial protonation on N5 via enol form 3, resulting in the reduction of dihydrofolate to tetrahydrofolate. In enzymatic mechanism, Asp-27 and the fixed water molecule H_2O-403 are probably indispensable to facilitate the keto/enol tautomerization.

In conclusion, pertinent to the previously purported enzymatic mechanism (14), as reproduced in Fig. 1(a), ab initio molecular orbital calculations support the keto/enol tautomerization mechanism. Enol form 3 is estimated to be comparably stable to keto form 1. Moreover, it is possible

that C6 carbon in enol form 3 could be activated for the nucleophilic hydride attack by the partial protonation on N5. Thus, the tautomerization of the pterin ring to enol form 3 is indispensable for enzymatic reduction of dihydrofolate by DHFR, since dihydrofolate is not protonated at the neutral pH region and Asp-27 is remote for direct proton transfer to imino N5. Although the details on the keto/enol tautomerization process is not clear, Asp-27 is supposed to take a predominant role in this process. The keto pterin ring could be reasonably converted to the enol pterin as shown in the mechanism illustrated in Fig. 1(a). The position of hydrogen between Asp-27 oxygen and N3 determines the partial positive charge on the pterin ring.

ACKNOWLEDGMENT

The generous allocation of computing time at the Research Information Processing System (RIPS) of AIST is greatly appreciated.

REFERENCES

1) Blakley, R. L. (1985) "Folates and Pterins", Blakley, R. L.; Benkovic, S. J., Eds., pp191-253. Wiley, New York.
2) Bolin, J. T., Filman, D. J., Matthews, D. A., Hamlin, R. C., Kraut, J. (1982) **J. Biol. Chem.**, 257, 13650-13662.
3) Filman, D. J., Bolin, J, T., Matthews, D. A., Kraut, J. (1982) **J. Biol. Chem.**, 257, 13663-13672.
4) Howell, E. E., Villafranca, J. E., Warren, M. S., Oatley, S. J., Kraut, J. (1986) **Science**, 231, 1123-1128.
5) Fontecilla-Camps, J. C., Bugg, C. E., Temple, Jr., C., Rose, J. D., Montgomery, J. A., Kisliuk, R. L. (1979) **J. Am. Chem. Soc**, 101, 6114-6115.
6) Hood, K., Robert, G. C. K., (1978) **Biochem. J.**, 171, 357-366.
7) Stone, S. R., Morrison, J. F., (1983) **Biochim. Biophys. Acta**, 745, 247-258.
8) Frisch, M. J., Binkley, J. S., Schlegel, H. B., Raghavachari, K., Melius, C. F., Martin, R. L., Stewart, J. J. P., Bobrowicz, F. W., Rohlfing, C. M., Kahn, L. R., Defrees, D. J. Seeger, R., Whiteside, R. A, Fox, D. J., Fleuder, E. M., Pople, J. A, (1984) Carnegie-Mellon Quantum Chemistry Publishing Unit, Pittsburgh PA.
9) Schlegel, H. B., Gund, P., Fluder, E. M., (1982) **J. Am. Chem. Soc.**, 104, 5347-5351.
10) Gready, J. E. (1985) **J. Am. Chem. Soc.**, 107, 6689-6695.
11) Gready, J. E. (1985) **Biochemistry**, 24, 4761-4766.
12) Gready, J. E. (1985) **J. Comput. Chem.**, 6, 377-400.
13) Ewig, C. S., Van Wazer, J. R. (1988) **J. Am. Chem. Soc.**, 110, 79-86.
14) Taira, K., Chen, J.-T., Fierke, C. A., Benkovic, S. J. (1987) **Bull. Chem. Soc. Jpn.**, 60, 3025-3030.