J Mol Model (2001) 7:103–111
DOI 10.1007/s008940100015

ORIGINAL PAPER

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Concerning the solvent effect in the tautomerism of uracil, 5-fluorouracil, and thymine by density-functional theory and ab initio calculations

Received: 11 September 2000 / Accepted: 16 February 2001 / Published online: 24 April 2001

Abstract The tautomerism of uracil, 5-fluorouracil, and thymine has been investigated in the gas phase and in solution. Electron correlation effects were included in ab initio computations at the MP2 level, and DFT calculations were performed using the B3LYP level. Full geometry optimizations were conducted at the HF/6-31G**, HF/6-31+G**, and B3LYP/6-31+G** levels. Single-point MP2/6-31+G** calculations were performed on the HF/6-31+G** optimized geometries. The influence of the solvent was examined from self-consistent reaction field calculations performed with ε=2.21 (1,4-dioxane) and ε=78.54 (water). The calculated relative free energies (ΔG) indicate that substitution of uracil at the position group does not change the relative free energy order of the uracil tautomers in the gas phase and in 1,4-dioxane (except at the MP2 level) whereas this ordering changes in water. Attachment of a fluorine atom changes the relative free energy order of uracil tautomers in the gas phase and in solution.

Keywords Ab initio · Solvent effect · Thymine · Uracil · 5-Fluorouracil

Introduction

The importance of tautomerism is crucial in biochemical and pharmacological research. Much experimental and theoretical work has been performed to investigate the rare tautomers of nucleic acid bases for their presumed crucial role in mutagenesis. Uracil and its derivatives are particularly interesting nucleic acid compounds. Both experimental and theoretical efforts have been directed toward determination of physicochemical properties of possible tautomeric forms. In an attempt to predict accurate energy differences between the various tautomers of uracil, 5-fluorouracil, and thymine in the gas phase, numerous quantum mechanical studies have been undertaken. Because these studies have been discussed many times, they are not reviewed in this work, although some will be mentioned to enable comparison with our results. Earlier theoretical studies of tautomeric reactions were essentially concerned with the gas phase. It is well known that the heterocyclic tautomerism depends on the environment [1]. Examination of the experimental data [2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16] strongly suggests that the dioxo-tautomers of uracil, 5-fluorouracil, and thymine are stable in the solid phase, in solution, in low temperature matrices, and in the vapor phase. Despite experimental predictions that the most stable tautomers are the dioxo form in solution, the relative stability of the rare tautomers is unclear. It has been difficult to study the tautomer structures experimentally because of the low occurrence of the rare tautomers. The improved accuracy and speed of computer-simulation techniques makes it practical to access such important, but experimentally inaccessible, energies and structural parameters accurately.

Uracil, 5-fluorouracil, and thymine can exist in the six tautomeric forms shown in Scheme 1. All nucleic acid bases can occur in a variety of tautomeric forms, differing in the positions of the protons. The occurrence of the rare tautomeric forms might lead to a point mutation [17, 18]. From the biological point of view, it is necessary to perform calculations in solution to understand the tautomerism of purine and pyrimidine bases. The aim of this work was to investigate the tautomerism of uracil in solution and to estimate the influence of the substituent effect on the tautomerization process for thymine and 5-fluorouracil. We have previously reported results for the tautomerism of 2-thiouracil obtained in the same fashion [19]. Extensive theoretical calculations have been performed on the energetic and structural preferences of uracil and its derivatives in the gas phase [20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45]. As far as we are aware the complete set of uracil, 5-fluorouracil, and thymine tautomers in solution with density functional theory and ab initio calculations has not previously been

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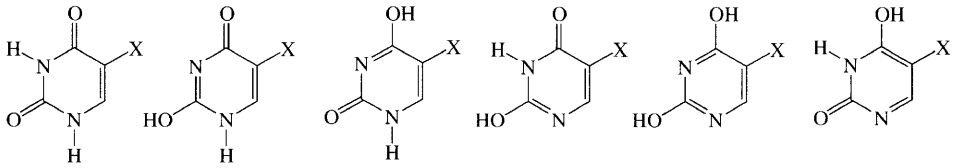
Scheme 1 The tautomeric forms of uracil, 5-fluorouracil, and thymine



X = H,	U	U1	U2	U3	U4	U5
X = F,	FU	FU1	FU2	FU3	FU4	FU5
X = CH_3,	T	T1	T2	T3	T4	T5

reported. Application of the SCRF method to the consideration of rare tautomeric forms in solution might provide useful information about the significance of tautomerism as a mechanism of mutation in nucleic acids. For this purpose, ab initio and density functional theory calculations have been used to examine the tautomeric preference of the title compounds.

Method

Full geometry optimizations of the six lowest energy tautomers of uracil, 5-fluorouracil, and thymine were performed at the HF/6-31G**, HF/6-31+G** and B3LYP/6-31+G** levels with the Gaussian 98 [46] package in the gas phase and in solution. Initially, all optimizations were performed at the HF/6-31G** level in the gas phase, and the resulting geometries were used as starting points in further calculations. The effects of electron correlation were taken into account by using Becke's three-parameter-hybrid (B3LYP) method in the density functional theory (DFT). An alternative approach to the computation of electron-correlation energy is to use second-order Møller–Plesset perturbation theory (MP2) as available in the Gaussian programs. Thus, single-point MP2 calculations were performed on the HF/6-31+G** optimized structures with the same basis set in the gas phase and in solution. Vibrational analysis was performed at all theoretical levels used here. Frequency calculations showed that all the tautomers were stationary points, and none showed imaginary frequencies in the vibrational analyses. ZPE (zero-point energy) values were obtained by scaling the calculated value by a factor of 0.9, which is a commonly accepted correction.

Solute–solvent interaction was evaluated by use of the SCRF method, which is based on Onsager's reaction field theory of electrostatic solvation [47]. All reaction field calculations were performed for ε=2.21 (1,4-dioxane) and ε=78.54 (water). The cavity radius values of uracil (a_0=3.86 Å), 5-fluorouracil (a_0=3.86 Å) and thymine (a_0=3.98 Å) were determined at the HF/6-31+G** level, using the volume=tight option implemented in Gaussian 98.

Results and discussion

It is well known that the DFT method demands less computational effort than ab initio molecular orbital calculations, and it has become an alternative to MP2. For this reason, the use of DFT methods has grown considerably in the last few years in many fields of chemistry. Here, we compare results for tautomerization energies obtained in different solutions for uracil, 5-fluorouracil, and thymine within the HF method and accounting for electron correlation using MP2 and DFT methods.

Theoretical studies of the tautomerization of pyrimidine bases are invaluable tools for analysis and prediction of their physicochemical properties. A previous ab initio study on 2-thiouracil [19] indicates that the order of stability of 2-thiouracil tautomers is very sensitive to the theoretical level and the solvent polarity. It was also found that the relative free energy changes (ΔG) should be taken into account in the order of stability of 2-thiouracil tautomers in the polar solvents. These results have raised the question of the relative order of stability of uracil, 5-fluorouracil, and thymine tautomers in solution. The self-consistent reaction field (SCRF) method has been found to be quite useful for accounting for solvent effects. Although specific hydrogen-bonding interactions between a solute and solvent are absent in the SCRF model, it is a computationally efficient method and simple to implement. For this reason, solvent effects have been introduced by the SCRF method. The energies have been calculated in self-consistent reaction fields of low (ε=2.21) and high (ε=78.54) dielectric constant.

One table in each of the appropriate sections shows the relative stability of the tautomers with respect to the most stable U, FU, and T forms. The free energy difference between the two tautomers, ΔG, was obtained by correcting ΔE with the zero-point vibrational energy difference (ΔZPE), the thermal correction difference (Δ(H–H_0)) and entropy difference (ΔS). All these correction terms were calculated using the HF/6-31+G** optimized geometries and given in the last table. The relationship between the relative free energy change (ΔG) and the computational level in water is shown graphically in three figures. Unless otherwise mentioned, our discussion will center on the relative free energy change values in this study. The geometrical parameters of uracil and derivatives have been discussed many times both experimentally and theoretically. We restrict this discussion to tautomerization energies only.

Uracil

The results of the calculations are presented in Tables 1 and 2. The relative energy order of uracil tautomers is the same as the relative free energy order in the gas phase and in solution, although these orders are different from each other at HF/6-31+G** and B3LYP/6-31+G** in water. As seen in the water solution results, the relative energy order of uracil tautomers is not changed on improvement of basis set quality, whereas it is changed by inclusion of electron correlation at the B3LYP level.

Table 1 Calculated energies[a] for uracil tautomers in the gas phase and in solution

ε	HF/6-31G**	HF/6-31+G**	B3LYP/6-31+G**	MP2/6-31+G**
U				
1	–412.481829	–412.493930	–414.847362	–413.688525
2.21	–412.484006	–412.496334	–414.849523	–413.690320
78.54	–412.487183	–412.499913	–414.852836	–413.692932
U1				
1	–412.448854	–412.461918	–414.816204	–413.658675
2.21	–412.453392	–412.466913	–414.820789	–413.662659
78.54	–412.460309	–412.474780	–414.828180	–413.668756
U2				
1	–412.460122	–412.472777	–414.828319	–413.670008
2.21	–412.462817	–412.475742	–414.830885	–413.672240
78.54	–412.466754	–412.480197	–414.834855	–413.675560
U3				
1	–412.463421	–412.475892	–414.829696	–413.672021
2.21	–412.464636	–412.477236	–414.830892	–413.672913
78.54	–412.466511	–412.479374	–414.832862	–413.674278
U4				
1	–412.461784	–412.474089	–414.826994	–413.671921
2.21	–412.461952	–412.474281	–414.827163	–413.672079
78.54	–412.462197	–412.474570	–414. 827423	–413.672314
U5				
1	–412.444787	–412.457088	–414.813278	–413.653955
2.21	–412.450508	–412.463257	–414.819003	–413.659058
78.54	–412.459360	–412.473128	–414.828529	–413.666985

[a] All energies in Hartrees

Table 2 Relative energies and free energies[a] for the six tautomeric forms of uracil in the gas phase and in solution

	U	U1	U2	U3	U4	U5
ε=1						
ΔE(HF/6-31G**)	0	20.69	13.62	11.5	12.58	23.24
ΔE(HF/6-31+G**)	0	20.09	13.27	11.32	12.45	23.12
ΔE(B3LYP/6-31+G**)	0	19.55	11.95	11.09	12.78	21.39
ΔE(MP2/6-31+G**)	0	18.73	11.62	10.36	10.42	21.69
ΔG(HF/6-31G**)	0	20.42	13.69	11.66	12.82	21.74
ΔG(HF/6-31+G**)	0	19.82	13.34	11.43	12.69	22.62
ΔG(B3LYP/6-31+G**)	0	19.28	12.02	11.20	13.02	20.89
ΔG(MP2/6-31+G**)	0	18.46	11.70	10.47	10.66	21.19
ε=2.21						
ΔE(HF/6-31G**)	0	19.21	13.30	12.15	13.84	21.02
ΔE(HF/6-31+G**)	0	18.46	12.92	11.98	13.84	20.76
ΔE(B3LYP/6-31+G**)	0	18.03	11.70	11.69	14.03	19.15
ΔE(MP2/6-31+G**)	0	17.36	11.35	10.92	11.45	19.62
ΔG(HF/6-31G**)	0	19.01	13.36	12.22	14.03	20.59
ΔG(HF/6-31+G**)	0	18.26	12.98	12.05	14.03	20.33
ΔG(B3LYP/6-31+G**)	0	17.83	11.76	11.76	14.22	18.72
ΔG(MP2/6-31+G**)	0	17.16	11.41	10.99	11.64	19.19
ε=78.54						
ΔE(HF/6-31G**)	0	16.86	12.82	12.97	15.68	17.46
ΔE(HF/6-31+G**)	0	15.77	12.37	12.89	15.90	16.81
ΔE(B3LYP/6-31+G**)	0	15.72	11.28	12.53	15.95	15.25
ΔE(MP2/6-31+G**)	0	15.17	10.90	11.71	12.94	16.28
ΔG(HF/6-31G**)	0	16.73	12.87	12.98	15.80	17.13
ΔG(HF/6-31+G**)	0	15.64	12.42	12.90	16.02	16.48
ΔG(B3LYP/6-31+G**)	0	15.59	11.33	12.54	16.07	14.92
ΔG(MP2/6-31+G**)	0	15.04	10.95	11.72	13.06	15.95

[a] Relative to U, and all energies in kcal mol^{-1}. HF/6-31+G** energies given in Table 7 were used in deriving ΔG values.

On the other hand, the relative free energy order of uracil tautomers is changed with the basis set and also inclusion of electron correlation at the B3LYP level. One cannot be certain if the energetic ordering of the species in question would not change if higher levels of theory were applied. The results demonstrate that inclusion of electron correlation at the MP2 level is unimportant in uracil tautomerization in the gas phase and in solution. This has also been found for the gas phase in previous theoretical studies [26, 29, 36, 40]. In this work we have confirmed these results for solution.

Clearly, U is the global minimum tautomer at all applied levels of theory in the gas phase and in solution. This is in accord with experimental studies [4, 5, 6, 7, 8, 9, 10, 11, 13, 15, 22] As seen in the gas phase results (Table 2), the order of stability of uracil tautomers using the ΔG values is U>U3>U4>U2>U1>U5 at the HF/6-31G**, HF/6-31+G**, and MP2/6-31+G** levels, whereas it is U>U3>U2>U4>U1>U5 at the B3LYP/6-31+G** level. Inclusion of electron correlation at the B3LYP level stabilizes tautomer U2 instead of U4. Scanlan et al. [20, 21] investigated the relative energy orders of uracil, 5-fluorouracil, and thymine tautomers at the HF/3-21G level in the gas phase and in aqueous solution. In contrast with our results, they concluded that substitution of the uracil 5-position by CH_3 or F does not change the order of stabilities of the tautomers in the gas phase, whereas the same orders change in aqueous solution. Also, the ab initio study of Leszczynski [36] showed that the relative free energy order for the four

Table 3 Calculated energies[a] for 5-fluorouracil tautomers in the gas phase and in solution

ε	HF/6-31G**	HF/6-31+G**	B3LYP/6-31+G**	MP2/6-31+G**
FU				
1	–511.314992	–511.331194	–514.073225	–512.692759
2.21	–511.316705	–511.333122	–514.075045	–512.694206
78.54	–511.319258	–511.336060	–514.077902	–512.696343
FU1				
1	–511.286096	–511.303265	–514.045990	–512.666572
2.21	–511.291237	–511.308982	–514.051284	–512.671113
78.54	–511.299195	–511.318115	–514.059975	–512.678105
FU2				
1	–511.291676	–511.308362	–514.053149	–512.672878
2.21	–511.293097	–511.309911	–514.054500	–512.674052
78.54	–511.295166	–511.312225	–514.056584	–512.675784
FU3				
1	–511.298973	–511.315797	–514.058602	–512.679511
2.21	–511.313061	–511.318105	–514.060691	–512.681110
78.54	–511.304195	–511.321795	–514.064183	–512.683550
FU4				
1	–511.290874	–511.307569	–514.050472	–512.673903
2.21	–511.291450	–511.308133	–514.051011	–512.674513
78.54	–511.292345	–511.309027	–514.051908	–512.675438
FU5				
1	–511.277963	–511.294834	–514.040803	–512.659479
2.21	–511.281577	–511.298663	–514.044467	–512.662764
78.54	–511.287228	–511.304839	–514.050631	–512.667860

[a] All energies in Hartrees

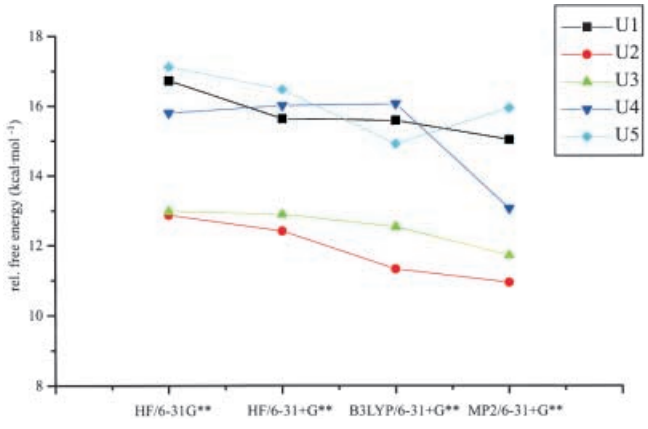


Fig. 1 Variation with computational level of the relative free energies (kcal mol^{-1}) of uracil tautomers in water

lowest energy tautomers of uracil is U>U3>U4>U2 (using the same notation as in this work), in agreement with our relative free energy order, at the MP2/6-31G** level in the gas phase.

Interestingly, the results obtained for 1,4-dioxane solution show that both the basis set and electron correlation effects at the B3LYP and MP2 levels are not important for the tautomerization of uracil. By comparing the data obtained in water (see Fig. 1), the relative free energy orders were found to be U>U2>U3>U1>U4>U5 and U>U2>U3>U5>U1>U4 at the HF/6-31+G** and B3LYP/6-31+G** levels, respectively. Tautomer U1 becomes more stable than U4 by 0.38 kcal mol^{-1} at the HF/6-31+G** level, and at the B3LYP/6-31+G** level, U5 becomes more stable than U1 and U4 by 0.67 and 1.15 kcal mol^{-1}, respectively. On the other hand, tautomer U2 is more stable than U3 by 0.11, 0.48, 1.21, and 0.77 kcal mol^{-1} at the HF/6-31G**, HF/6-31+G**, B3LYP/6-31+G** and MP2/6-31+G** levels, respectively. In all cases except for the HF/6-31+G** (ε=78.54) and B3LYP/6-31+G** (ε=78.54) levels, U1 and U5 are the least stable structures. At all studied levels of theory, U3 is the second most stable form in the gas phase and in 1,4-dioxane, whereas U2 is the second most stable tautomer, instead of U3, in water.

5-Fluorouracil

The energies and relative free energies for tautomers of 5-fluorouracil are reported in Tables 3 and 4. The stability difference relative to the other tautomers is so large as to guarantee that FU is the only important tautomer in the gas phase and in solution, as previously suggested both theoretically [21, 29, 40, 48] and experimentally [49, 50]. The next most stable tautomer is FU3 at all levels in the gas phase and in solution, but FU1 at the MP2/6-31+G** level in water. In all cases the presence of the fluorine atom at position 5 does not increase the stability of the FU5 form.

The relative free energy order for 5-fluorouracil tautomers is similar to the relative energy order, except the HF/6-31G** (ε=2.21) and MP2/6-31+G** (ε=78.54) results, in the gas phase and in solution. Considering the

Table 4 Relative energies and free energies[a] for the six tautomeric forms of 5-fluorouracil in the gas phase and in solution

	FU	FU1	FU2	FU3	FU4	FU5
ε=1						
ΔE(HF/6-31G**)	0	18.13	14.63	10.05	15.13	23.24
ΔE(HF/6-31+G**)	0	17.53	14.33	9.66	14.82	22.82
ΔE(B3LYP/6-31+G**)	0	17.09	12.60	9.18	14.28	20.35
ΔE(MP2/6-31+G**)	0	16.43	12.48	8.31	11.83	20.88
ΔG(HF/6-31G**)	0	17.95	14.63	10.26	15.20	22.69
ΔG(HF/6-31+G**)	0	17.35	14.33	9.87	14.89	22.27
ΔG(B3LYP/6-31+G**)	0	16.91	12.60	9.39	14.35	19.80
ΔG(MP2/6-31+G**)	0	16.25	12.48	8.52	11.90	20.33
ε=2.21						
ΔE(HF/6-31G**)	0	15.98	14.81	2.29	15.85	22.04
ΔE(HF/6-31+G**)	0	15.15	14.57	9.42	15.68	21.62
ΔE(B3LYP/6-31+G**)	0	14.91	12.89	9.01	15.08	19.19
ΔE(MP2/6-31+G**)	0	14.49	12.65	8.22	12.36	19.73
ΔG(HF/6-31G**)	0	15.87	14.80	2.45	15.89	21.54
ΔG(HF/6-31+G**)	0	15.04	14.56	9.58	15.72	21.10
ΔG(B3LYP/6-31+G**)	0	14.80	12.88	9.17	15.12	18.67
ΔG(MP2/6-31+G**)	0	14.38	12.64	8.38	12.40	19.21
ε=78.54						
ΔE(HF/6-31G**)	0	12.59	15.12	9.45	16.89	20.10
ΔE(HF/6-31+G**)	0	11.26	14.96	8.95	16.96	19.59
ΔE(B3LYP/6-31+G**)	0	11.25	13.38	8.61	16.31	17.11
ΔE(MP2/6-31+G**)	0	11.44	12.90	8.03	13.12	17.87
ΔG(HF/6-31G**)	0	12.55	15.11	9.56	16.91	19.62
ΔG(HF/6-31+G**)	0	11.22	14.95	9.06	16.98	19.11
ΔG(B3LYP/6-31+G**)	0	11.21	13.37	8.72	16.33	16.63
ΔG(MP2/6-31+G**)	0	11.40	12.89	13.23	13.14	17.39

[a] Relative to FU, and all energies in kcal mol^{-1}. HF/6-31+G** energies given in Table 7 were used in deriving ΔG values

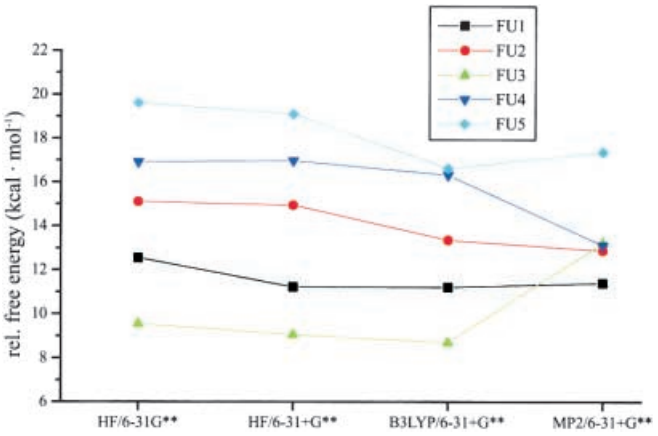


Fig. 2 Variation with computational level of the relative free energies (kcal mol^{-1}) of 5-fluorouracil tautomers in water

ΔG values, we note the that FU1 tautomer is stabilized at the HF/6-31G** (ε=2.21) and MP2/6-31+G** (ε=78.54) levels, but FU4 and FU3 are destabilized at the same levels. Improvement of the basis set at the HF level and inclusion of the electron correlation at the B3LYP level in the gas phase and in solution do not change the relative free energy orders of 5-fluorouracil tautomers, whereas contributions from electron correlation computed using MP2 at the HF optimized molecular geometries change these orders in the gas phase and in solution. Inclusion of electron correlation (MP2/6-31+G**) shows that FU4 is more stable than FU2 by 0.58 and 0.24 kcal mol^{-1} in the gas phase and in 1,4-dioxane, respectively. At the MP2/6-31+G** level, the relative free energy of the fifth most stable tautomer of FU3 amounts to only 13.23 kcal mol^{-1}, whereas it is the second most stable tautomer with relative free energies of 9.56, 9.06, and 8.72 kcal mol^{-1} at the HF/6-31G**, HF/6-31+G** and B3LYP/6-31+G** levels, respectively. The electron-correlation energy contributions introduced at the MP2/6-31+G** level in water further stabilize tautomers FU1, FU2, and FU4. Comparing the results for uracil obtained in water with those obtained for 5-fluorouracil, we find that the relative free energy order of uracil tautomers is hardly affected by substitution with the fluorine atom. This substitution stabilizes the FU1 and FU3 tautomers. Leś et al. [29] studied the three lowest energy tautomeric forms of uracil and 5-fluorouracil using second-order many-body perturbation theory (MBPT (2)) with Gaussian DZP basis sets in the gas phase. In both cases the relative free energy orders were found to be U>U3>U2 and FU>FU3>FU2. Our results in Table 4 indicate that solvation changes the preference between tautomers. Marino et al. [48] have investigated the four lowest energy tautomers of 5-fluorouracil by using the linear combination of Gaussian-type orbital non-local spin density (LCGTO-NLSD) method with a Gaussian DZVP basis set and employing different exchange-correlation functionals to take into account the non-local corrections in the gas phase and in aqueous solution. They concluded that 5-fluorouracil tautomers obey the stability sequence: FU>FU3>FU2>FU4 (using the same notation as in this work) and that the presence of water does not affect the relative stabilities found in the gas phase. In this work, the predicted order of stability of 5-fluorouracil tautomers at all levels except MP2/6-31+G**, using the relative free energies, was found to be FU>FU3>FU2>FU4>FU1>FU5, FU>FU3>FU2>FU1>FU4>FU5 and FU>FU3>FU1>FU2>FU4>FU5 in the gas phase, in 1,4-dioxane, and in water (see Fig. 2), respectively. It is seen that FU1 becomes more stable than FU4 on going from the gas phase to a polar environment. Upon comparing our results with Marino's results [48] for 5-fluorouracil tautomerization one notices that the relative free energy orders of uracil and derivatives of tautomers can be changed with the considered tautomer numbers.

Thymine

The energies and free energy differences relative to the T tautomer at the different levels of theory for the six tautomers of thymine are given in Tables 5 and 6. All the theoretical calculations suggest a higher T tautomer energetic stability, in agreement with experimental observations [2, 3, 9, 15]. Considering the results obtained here, the second most stable form is T3 in the gas phase

Table 5 Calculated energies[a] for thymine tautomers in the gas phase and in solution

ε	HF/6-31G**	HF/6-31+G**	B3LYP/6-31+G**	MP2/6-31+G**
T				
1	–451.524188	–451.535778	–454.170253	–452.878459
2.21	–451.526049	–451.537879	–454.172175	–452.880360
78.54	–451.528761	–451.540998	–454.175113	–452.883239
T1				
1	–451.492228	–451.504762	–454.140139	–452.849560
2.21	–451.495848	–451.508719	–454.143762	–452.853192
78.54	–451.501345	–451.514896	–454.149543	–452.858951
T2				
1	–451.500629	–451.512865	–454.149922	–452.858877
2.21	–451.503343	–451.515961	–454.152696	–452.861620
78.54	–451.507418	–451.520743	–454.157143	–452.865988
T3				
1	–451.505630	–451.517621	–454.152849	–452.862552
2.21	–451.506465	–451.518513	–454.153621	–452.863249
78.54	–451.507762	–451.519935	–454.154896	–452.864400
T4				
1	–451.502281	–451.514280	–454.149067	–452.861712
2.21	–451.502508	–451.514566	–454.149352	–452.862057
78.54	–451.502851	–451.515007	–454.149813	–452.862607
T5				
1	–451.477466	–451.489595	–454.128945	–452.836654
2.21	–451.481095	–451.493585	–454.132851	–452.840493
78.54	–451.486762	–451.500030	–454.139389	–452.846977

[a] All energies in Hartrees

and in 1,4-dioxane solution and also at the HF/6-31G** level in aqueous solution. The relative energy order and the relative free energy order of the thymine tautomers are the same, except for HF/6-31+G** (ε=78.54) results, at all levels in the gas phase and in solution. Considering the ΔG values at the HF/6-31+G** (ε=78.54) level leads to significant stabilization of the T1 tautomer. From Table 6 it is clear that improving the basis set quality does not change the order of stability of thymine tautomers in the gas phase and in 1,4-dioxane. Electron-correlation energy contributions (MP2/6-31+G** level) in the gas phase maintain the order of stability of thymine tautomers T>T3>T4>T2>T1>T5 with relative free energies equal to 0, 10.09, 10.64, 12.39, 17.91, and 24.47 kcal mol^{-1}, respectively. Electron-correlation energy contributions at the B3LYP/6-31+G** level provide additional stability for the T2 tautomer, but destabilize T4 in the gas phase. That is, T2 is more stable than T4 by 0.56 kcal mol^{-1}. Ha et al. [37] reported the optimized structures and electronic energies for all geometric isomers of all five tautomeric forms of thymine using HF/6-31G** and MP2 HF/6-31G** computations in the gas phase. They found the same energy ordering as for our the HF/6-31G** gas phase results.

Interestingly, our results for 1,4-dioxane indicate that the order of stability of thymine tautomers does not change at the B3LYP level, but does do so at the MP2 level, in contrast with the gas-phase results. The T4 tautomer is calculated to be more stable than T2 by 0.29 kcal mol^{-1} upon inclusion of correlation energy at the MP2 level in 1,4-dioxane. The same relative free energy order has been found for uracil and thymine tautomers except the MP2 (ε=2.21) results for thymine tautomers, at all levels in the gas phase and in 1,4-dioxane. In aqueous solution the orders of stability are different for the tautomers of uracil, 5-fluorouracil, and thymine.

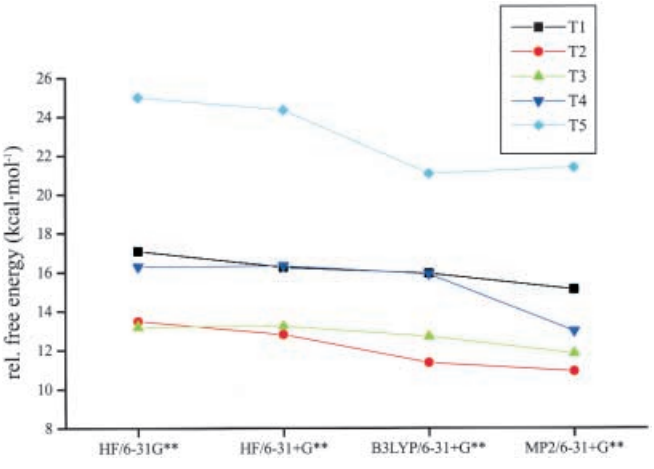


Fig. 3 Variation with computational level of the relative free energies (kcal mol^{-1}) of thymine tautomers in water

According to our results for aqueous solution, basis set and electron-correlation energy contributions have an effect on the order of stability of thymine tautomers. Improvement of the basis set and inclusion of the electron-correlation energies favor the T2 form over T3 by 0.44, 1.34, and 0.92 kcal mol^{-1} at the HF/6-31+G**, B3LYP/6-31+G** and MP2/6-31+G** levels, respec-

Table 6 Relative energies and free energies[a] for the six tautomeric forms of thymine in the gas phase and in solution

	T	T1	T2	T3	T4	T5
ε=1						
ΔE(HF/6-31G**)	0	20.06	14.78	11.65	13.75	29.32
ΔE(HF/6-31+G**)	0	19.46	14.38	11.39	13.49	28.98
ΔE(B3LYP/6-31+G**)	0	18.90	12.76	10.92	13.29	25.92
ΔE(MP2/6-31+G**)	0	18.13	12.29	9.98	10.51	26.23
ΔG(HF/6-31G**)	0	17.84	14.88	11.76	13.88	27.56
ΔG(HF/6-31+G**)	0	19.24	14.48	11.50	13.62	27.22
ΔG(B3LYP/6-31+G**)	0	18.68	12.86	11.03	13.42	24.16
ΔG(MP2/6-31+G**)	0	17.91	12.39	10.09	10.64	24.47
ε=2.21						
ΔE(HF/6-31G**)	0	18.95	14.25	12.29	14.77	28.21
ΔE(HF/6-31+G**)	0	18.30	13.75	12.15	14.63	27.79
ΔE(B3LYP/6-31+G**)	0	17.83	12.22	11.64	14.32	24.68
ΔE(MP2/6-31+G**)	0	17.05	11.76	10.74	11.49	25.02
ΔG(HF/6-31G**)	0	18.77	14.34	12.35	14.84	26.65
ΔG(HF/6-31+G**)	0	18.12	13.84	12.21	14.70	26.23
ΔG(B3LYP/6-31+G**)	0	17.65	12.31	11.70	14.39	23.12
ΔG(MP2/6-31+G**)	0	16.87	11.85	10.80	11.56	23.46
ε=78.54						
ΔE(HF/6-31G**)	0	17.20	13.39	13.18	16.26	26.35
ΔE(HF/6-31+G**)	0	16.38	12.71	13.22	16.31	25.71
ΔE(B3LYP/6-31+G**)	0	16.05	11.28	12.69	15.88	22.42
ΔE(MP2/6-31+G**)	0	15.24	10.83	11.82	12.95	22.75
ΔG(HF/6-31G**)	0	17.08	13.47	13.19	16.28	25.00
ΔG(HF/6-31+G**)	0	16.26	12.79	13.23	16.33	24.36
ΔG(B3LYP/6-31+G**)	0	15.93	11.36	12.70	15.90	21.07
ΔG(MP2/6-31+G**)	0	15.12	10.91	11.83	12.97	21.40

[a] Relative to T, and all energies in kcal mol^{-1}. HF/6-31+G** energies given in Table 7 were used in deriving ΔG values

Table 7 Zero-point energies (ZPE), entropies (S), thermal corrections ($H–H_o$) and dipole moments[a] for tautomers of uracil, 5-fluorouracil, and thymine in the gas phase and in solution at 25 °C[b]

		U	U1	U2	U3	U4	U5
ε=1	ZPE	59.04	58.78	59.02	59.01	59.04	58.50
	S	77.70	77.87	77.15	76.96	76.41	77.64
	$H-H_0$	4.24	4.28	4.17	4.16	4.10	4.26
	μ	4.94	7.05	5.48	3.65	1.40	7.80
ε=2.21	ZPE	59.03	58.81	59.00	58.96	58.99	58.54
	S	77.61	77.62	77.08	76.92	76.45	77.37
	$H-H_0$	4.23	4.25	4.16	4.16	4.11	4.22
	μ	5.42	7.91	6.04	4.11	1.53	8.81
ε=78.54	ZPE	58.99	58.81	58.95	58.87	58.91	58.55
	S	77.49	77.33	77.01	76.85	76.48	77.00
	$H-H_0$	4.21	4.21	4.16	4.15	4.11	4.17
	μ	6.15	9.27	6.88	4.85	1.75	10.44
		FU	FU1	FU2	FU3	FU4	FU5
ε=1	ZPE	53.64	53.44	53.56	53.68	53.45	53.07
	S	82.13	82.10	81.63	81.24	80.86	82.16
	$H-H_0$	4.77	4.78	4.70	4.67	4.65	4.80
	μ	4.42	7.55	3.98	4.80	2.37	6.17
ε=2.21	ZPE	53.62	53.46	53.53	53.62	53.41	53.06
	S	82.03	81.82	81.58	81.18	80.86	81.97
	$H-H_0$	4.75	4.74	4.70	4.66	4.65	4.77
	μ	4.90	8.51	4.37	5.41	2.67	6.98
ε=78.54	ZPE	53.57	53.43	53.48	53.51	53.36	53.02
	S	81.91	81.48	81.52	81.08	80.86	81.66
	$H-H_0$	4.73	4.70	4.69	4.65	4.65	4.73
	μ	5.63	10.08	4.97	6.40	3.14	8.31
		T	T1	T2	T3	T4	T5
ε=1	ZPE	77.55	77.29	77.54	77.51	77.48	76.46
	S	85.08	85.02	84.49	84.34	84.05	88.47
	$H-H_0$	5.24	5.26	5.17	5.17	5.13	5.58
	μ	4.86	6.60	5.85	3.12	1.78	6.59
ε=2.21	ZPE	77.54	77.31	77.52	77.46	77.43	76.48
	S	85.01	84.83	84.42	84.33	84.08	87.70
	$H-H_0$	5.23	5.23	5.16	5.17	5.13	5.53
	μ	5.33	7.38	6.52	3.52	1.98	7.46
ε=78.54	ZPE	77.51	77.32	77.48	77.39	77.37	76.48
	S	84.95	84.60	84.36	84.29	84.12	86.76
	$H-H_0$	5.23	5.20	5.16	5.16	5.14	5.45
	μ	6.03	8.61	7.56	4.17	2.30	8.90

[a] HF/6-31+G** values in Debye
[b] All energy terms based on the HF/6-31+G** optimized geometries. ZPE and $H–H_o$ in kcal mol^{-1}, S in cal mol^{-1} K^{-1}

tively. Additionally, the T1 form is favored over T4 by 0.07 kcal mol^{-1} at the HF/6-31+G** level. Single-point MP2 calculations of relative free energy ordering are similar to those obtained at the B3LYP level. At these levels, the relative free energy order was found to be T>T2>T3>T4>T1>T5 (see Fig. 3). An ab initio study of Kwiatkowski et al. [26] suggests that electron correlation is not important in estimating relative stabilities of tautomers. The results of this study indicate that electron-correlation energies at the B3LYP and MP2 levels are important in the relative stabilities of uracil, 5-fluorouracil, and thymine tautomers in the gas phase and in solution.

Dipole moments

Table 7 shows dipole moments of the species studied in different media. The interaction between different tautomers and a polar environment is correlated with the magnitude of the solute dipole moment. It is expected that the tautomer with the largest dipole moment should become more stable than others with relatively small dipole moments on going to polar solvent. There is no direct correlation between dipole moment and relative stability in the SCRF calculations. For example, the U4 form, which has the lowest dipole moment, is the fifth most stable, and U5, which has the highest dipole moment, is the least stable tautomer at the HF/6-31+G** level in water.

The calculated values of 4.94 D (ε=1) and 5.42 D (ε=2.21) for the U tautomer are significantly larger than the experimental dipole moments of 3.87 D [6] and 4.16 D [51] in the gas phase and in 1,4-dioxane solution, respectively. This overestimation indicates the limitations of the HF geometry optimizations. Similar results were reported by Leszczynski [36]. It has, on the other hand, been pointed out in various studies that the calculated dipole moments show little sensitivity to the applied levels [36, 40, 52, 53]. Note that there is substantial

enhancement of the dipole moments for all the species considered on going from the gas phase to polar solution. Of all the compounds the four tautomeric forms are characterized by the smallest calculated dipole moments. The magnitude of the dipole moment of U, the most stable tautomer of uracil, is larger than that of the 5-fluorouracil and thymine tautomers. Substitutions at the 5-position of the U1 and U3 forms of uracil causes an increase in the dipole moments of the corresponding tautomers in 5-fluorouracil, and a decrease in thymine.

Experimental dipole moment values for thymine in 1,4-dioxane range between 3.95 and 4.20 D [51, 54]. Very recently, Šponer et al. [55] reported the dipole moment of thymine to be 4.01 D at the MP2/6-31G* level in the gas phase. In this work the calculated dipole moment of thymine at the HF level (4.86 D in the gas phase and 5.33 D in 1,4-dioxane) is larger than the experimental and theoretical results. For 5-fluorouracil there are no previous experimental dipole moment values with which to compare our results. Scanlan et al. [21] have calculated the dipole moment of 5-fluorouracil to be 4.30 at the HF/3-21G level in the gas phase. Marino et al. [48] found the dipole moment of 5-fluorouracil to be 4.07, 4.14, and 4.13 D at PP, BP and BP-LYP levels, respectively. Our gas phase value is 4.42 D.

Conclusions

The major conclusions to be gleaned from this work are:

1. Considering the ΔG values, in the gas phase and in solution, the dioxo-tautomers of uracil, 5-fluorouracil, and thymine (U, FU, and T) are the most stable structures in agreement with the experimental data. For all systems except uracil at the B3LYP level (ε=78.54), the five forms are the least stable tautomers.
2. In the gas phase, substitution of uracil by CH_3 at the 5-position does not change the order of stability of the tautomers, whereas attachment of an F atom changes the order of stability at the B3LYP and MP2 levels. Considering the solvent causes reordering of the orders of stability of uracil, 5-fluorouracil, and thymine tautomers. Additionally, substituents at the 5 position of uracil lead to changes in the free energy of tautomerization in solution. It can be expected that by varying solvent polarity one might force the presence of a particular tautomeric form.
3. In general the MP2 and DFT (B3LYP) relative free energies are smaller than the corresponding HF values. The order of stability is very sensitive to the level of theory and environment. We find that, in contrast with the results obtained for uracil in this paper, correlation effects at the MP2 level cannot be ignored in the prediction of the order of stability of tautomeric forms of 5-fluorouracil in the gas phase and in solution. For thymine the MP2 method changes the order of stability of tautomers in solution only. The DFT method, on the other hand, does not affect the relative stabilities of the 5-fluorouracil tautomers in the gas phase and in solution, whereas the same method is important in uracil and thymine tautomerization in the gas phase and in water.
4. Relative free energy changes (ΔG) should be taken into account in the prediction of the order of stability of uracil and its derivatives in solution. Similar conclusions were drawn from our previous calculations on 2-thiouracil tautomerization [19].
5. There are currently no data on the relative energies of the complete tautomers of uracil, 5-fluorouracil, and thymine in solution; thus our calculations provide a prediction of the relative energies for these compounds.

Acknowledgement We thank Cumhuriyet University Research Funds for providing the program Gaussian 98 W.

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