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Solvent effect on the conformation of the Hoechst 33258 agent

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Abstract

The conformational preferences of the Hoechst 33258 agent were studied with ab initio, semiempirical molecular orbital, electrostatic calculations and by Monte Carlo free energy simulations with explicit solvent representation using the adaptive umbrella sampling method. The semiempirical approach to calculating the solvation free energies was found in qualitative agreement with the Monte Carlo result. The optimal conformation in the solvent was found to be close to the previously calculated optimal conformation for binding. © 1997 Elsevier Science B.V.

Keywords: Hartree–Fock; Amsol; Monte Carlo; Adaptive umbrella sampling; Hoechst 33258 agent

1. Introduction

Hoechst 33258 is a synthetic N-methylpiperazine derivative, developed by the Hoechst Pharmaceutical Company. It does not show anticancer activity but it is effective as an anthelminthic agent [1,2]. The Hoechst agent belongs to a group of molecules which bind to the minor groove of B-DNA. These molecules feature a similar basic structure: a repetition of a structural motif that produces an arc-like conformation, matching the turn of the B-DNA helix. There are two main categories of these compounds: the oligopeptides called "lexitropsins" among which are netropsin, distamycin and others, and the Hoechst agent with its analogs. The first category has been shown to exhibit antitumor activity [3,4].

A majority of lexitropsins as well as the Hoechst agents feature cationic groups either at one end or at both, fact which facilitates the binding to the negative potential in the minor groove of DNA.

Hoechst 33258, shown in Fig. 1 has been geometry-optimized at the Hartree–Fock level with a fragment-wise procedure using the 3-21G and the STO-3G basis sets, as implemented by the GAUSSIAN 92 computer program [5]. The optimum geometry thus obtained and details of the optimization are reported in Ref. [6]. These calculations refer to a gas-phase compound, without the presence of a solvent. The insertion of lexitropsins or of the Hoechst agent into the minor groove of B-DNA is supposed to occur via the displacement of the water molecules present in the groove. The compound has to adopt the best DNA binding geometry, which, as shown previously [6] is not very different from the optimum geometry of the molecule. However, before the drug inserts itself into the minor groove, it is found in an aqueous solution

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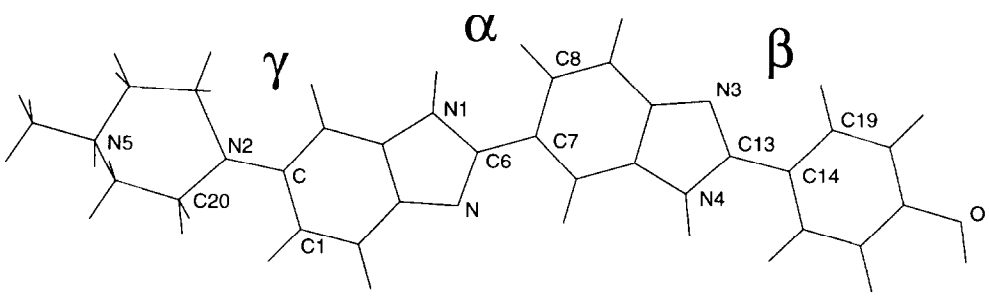

Fig. 1. The Hoechst 33258 molecule in the $\alpha = \beta = \gamma = 0°$ conformation.

and it is interesting to find out how different is the geometry of the molecule in solution from the one in gas phase. In order to accomplish this, the solvent effect on various conformations of the drug has to be calculated and the stabilizing energy of the solvent has to be compared to the differences between the energies of these conformations in gas phase. This way, the most stable conformation in solvent can be identified.

To distinguish the various conformations, three parameters have been considered for a series of single-point energy calculations.

The angle α ($C_8C_7C_6N_1$), which is the torsion angle between the two central benzimidazole rings, the angle β ($C_{19}C_{14}C_{13}N_3$), which is the torsion angle between the plane of the benzimidazole ring attached to the phenol moiety and the plane of the phenol ring, and the angle γ ($C_{20}N_2CC_1$), which is the angle defined by the plane of the benzimidazole ring attached to the piperazine group and the $C_{20}N_2C$ plane. The other parameters of the molecule have been kept frozen at the values obtained and reported previously [6].

2. Methods

The conformational preferences of the Hoechst agent were studied by three different methods: ab initio calculations, semiempirical calculations, both with empirical solvation contribution and without; calculations of the electrostatic contributions and free energy simulations of the Hoechst agent immersed in explicit solvent.

Ab initio calculations were performed with the STO-3G basis set at the Hartree–Fock level using the GAUSSIAN 92 program [5]. Semiempirical molecular orbital calculations were performed using the AM1 parametrization [7]. The solvation free energy was estimated using the approach of Cramer and Truhlar [8] as realized in the AMSOL 3.5 program [9]. These calculations first obtain the electronic and nuclear energy from semiempirical molecular orbital calculations referred to above. The molecular orbital calculations also provided atomic (partial) charges to be used (via Mulliken population analysis) in the reaction field calculations that provide the electrostatic contribution to the solvation. Cavity and dispersion effects are incorporated via surface-dependent empirical contribution with coefficients parametrized for several atom types, optionally dependent on the environment, called SM1 or SM1a, respectively. Since we studied only conformational changes we used the SM1a parametrization.

The solvation free energy was also estimated from a purely electrostatic model where the solvent was represented by a dielectric continuum and the electrostatic interaction energy of the solute was obtained by solving the Poisson–Boltzmann equation using finite differences (FDPB) as implemented in the DELPHI program [10]. On small neutral organic molecules this approach was found to give the electrostatic contribution to the solvation free energies in good correlation with values calculated from free energy simulations [11]. The same comparison for ions showed large differences. A comparison of Amsol/SM1 and FDPB results on a variety of neutral and ionic compounds [12] found good correlation for the ionic compounds, not so good for the neutral ones. Note that this does not contradict the previous findings since there only the electrostatic contributions were considered. Atomic partial charges in our calculations

used the values obtained from the gas phase ab initio calculations described earlier using Mulliken population analysis. The internal dielectric constant was set to 2 and the external dielectric constant was set to 80. The molecule filled 23% of a box that was divided into 65 grids along each coordinate axis and 3-step focusing boundary conditions were applied.

We also performed free energy simulations in explicit solvent along a few degrees of freedoms. Apart from the selected torsion angles, all intramolecular parameters of the solute were held constant. Also, as the solute's intramolecular energy was not calculated during the simulation the calculated free energy profile contains the solvent's contribution only although there is a small contribution from the solute due to the fact that the partial charges were varied with the conformation. Since the mean change in charge was 0.002 electron and the largest change was 0.04 electron, this solute contribution is expected to be small. These calculations are generally orders of magnitude more time consuming [13,14] than either AMSOL or DELPHI runs thus the scope of the explicit solvent calculations was limited relative to the semi-empirical or electrostatic calculations.

The free energy simulations sampled the space $0° \leq \alpha \leq 180°$ (with $\beta = \gamma = 0°$), $0° \leq \beta \leq 90°$ (with $\alpha = 90°$, $\gamma = 0°$) and $0° \leq \gamma \leq 90°$ (with $\alpha = 90°$, $\beta = 0°$). The solute–solvent interactions were described with the Amber force field [15] using partial charges obtained by Mulliken population analysis of the HF calculations. For intermediate torsion angles the partial charges were obtained from linear interpolation. The water–water interactions used the TIP3P model [16]. The free energy profile (i.e., the potential of mean force, $W(\phi)$) along these torsion angles were obtained using the adaptive umbrella sampling technique [17–20] that determines the PMF in an iterative fashion. Each iteration provides an estimate of the PMF based on the frequency distribution of sampling

Table 1
Conformational energies free energies calculated

α	β	γ	E_{HF}	E_{AM1}	E_{Amsol}	E_{POL}	E_{CAV}	$E_{El.stat.}$
0	0	0	− 844501.31	433.80	370.72	− 90.79	− 2.02	− 31.90
0	0	0	0.00	0.00	0.00	0.00	0.00	0.00
30	0	0	0.69	− 0.15	− 0.48	0.00	− 0.35	− 0.04
60	0	0	4.19	0.75	0.25	0.11	− 0.53	− 0.10
90	0	0	6.63	1.81	1.47	0.16	− 0.35	− 0.13
120	0	0	4.88	0.92	1.06	0.34	0.04	− 0.05
180	0	0	1.94	0.73	0.68	− 0.13	0.00	0.18
0	0	90	1.06	2.88	5.72	3.80	0.67	0.04
30	0	90	1.88	2.85	5.27	3.64	0.32	0.03
60	0	90	5.50	3.83	5.99	3.56	0.15	− 0.06
90	0	90	9.06	4.89	7.25	3.64	0.32	− 0.10
120	0	90	8.00	3.96	6.87	3.87	0.71	0.03
180	0	90	6.19	3.74	6.49	3.49	0.67	0.22
0	90	0	3.19	0.50	0.87	− 0.02	0.39	− 0.32
30	90	0	7.13	0.32	0.19	− 0.13	− 0.05	− 0.27
60	90	0	7.69	1.18	0.98	0.06	− 0.19	− 0.34
90	90	0	11.88	2.22	2.34	0.29	0.03	− 0.35
120	90	0	11.13	1.33	1.73	0.25	0.36	− 0.24
180	90	0	13.63	1.19	1.39	− 0.20	0.30	0.11
60	90	90	12.56	0.50	0.87	− 0.02	0.39	

Energies are in kcal mol^{-1}.

In the first row the calculated absolute energies are given while in subsequent rows the values in the first row are subtracted to provide the changes with the variation of the conformation.

E_{HF} is the total electronic nuclear energy of the molecule calculated in the Hartree–Fock approximation while E_{AM1} and E_{Amsol} represent heats of formation calculated with the AM1 and SM1a parametrizations, respectively.

E_{POL} and E_{CAV} are the polarization and surface free energies calculated by AMSOL and $E_{El.\ stat.}$ is the electrostatic solvation energy computed by DELPHI.

$P(\phi)$:

$$W(\phi) = -kT \ln P(\phi) + \text{Const.}$$

This estimate is combined with the estimate of previous iterations to yield eventually the full $W(\phi)$.

The simulations used the force-biased [21] Metropolis Monte Carlo [22] method with preferential sampling [23,24] applied to the solvent based on the nearest solute heavy atom [25]. The Hoechst agent molecule was surrounded by 666 water molecules in a hexagonal prism and periodic boundary conditions were applied to model the condensed phase. The calculations were broken down into 30° intervals (''windows''). Each iteration consisted of 4×10^5 Monte Carlo steps (attempted displacement) and each window involved 2.4×10^7 Monte Carlo steps for the PMF along α and 8×10^6 steps for the PMFs along the β and γ angles.

3. Results

Table 1 displays the change in the energy of the molecule as a function of the angles α, β, and γ, calculated using the ab initio Hartree–Fock method, the semiempirical AM1 method as well as the solvation energies calculated with the semiempirical Amsol/SM1a method and the electrostatic energies, respectively. The first row of the table provides the absolute energy values at $\alpha = \beta = \gamma = 0°$ and successive lines give the change in energies compared to that conformation. Note that the electrostatic calculations give only the solvent's contribution to the solvation free energy, while the Amsol/SM1a results include both the solute's intramolecular as well as the solvent's contribution to the solvation free energy.

Table 2 displays estimates of the change in solvation free energy as the conformation is varied. The column headed by $E_{\text{Amsol}} - E_{\text{AM1}}$ gives the variation in the Amsol/SM1a-AM1 energy difference – this is the straightforward semiempirical estimate. We also provided the variation of the Amsol/SM1a-HF energy difference in a column headed by $E_{\text{Amsol}} - E_{\text{HF}}$. Since the type of approximations made by the semiempirical and the Hartree–Fock procedure are rather different, this quantity is of limited significance, though. The change in the solvation free energy estimated by the Monte Carlo simulations of explicitly solvated system

Table 2
Solvation free energy change estimates

α	β	γ	$E_{\text{Amsol}} - E_{\text{AM1}}$	$E_{\text{Amsol}} - E_{\text{HF}}$	E_{MC}
0	0	0	0.00	0.00	0.00
30	0	0	− 0.32	− 1.16	1.08
60	0	0	− 0.50	− 3.94	− 0.22
90	0	0	− 0.34	− 5.16	− 1.10
120	0	0	0.14	− 3.82	− 1.64
180	0	0	− 0.06	− 1.26	− 2.12
0	0	90	2.84	4.66	
30	0	90	2.42	3.40	
60	0	90	2.16	0.49	
90	0	90	2.36	− 1.81	0.54
120	0	90	2.91	− 1.13	
180	0	90	2.75	0.31	
0	90	0	0.37	− 2.32	
30	90	0	− 0.13	− 6.93	
60	90	0	− 0.21	− 6.71	
90	90	0	0.12	− 9.54	0.34
120	90	0	0.39	− 9.40	
180	90	0	0.20	− 12.23	
60	90	90	0.37	− 11.69	

Energies are in kcal/mol^{-1}.
The energies of the $\alpha = \beta = \gamma = 0°$ were set to zero.
Amsol energies were calculated with the SM1a parametrization. Hartree–Fock (HF) energies were calculated at the STO-3G level.

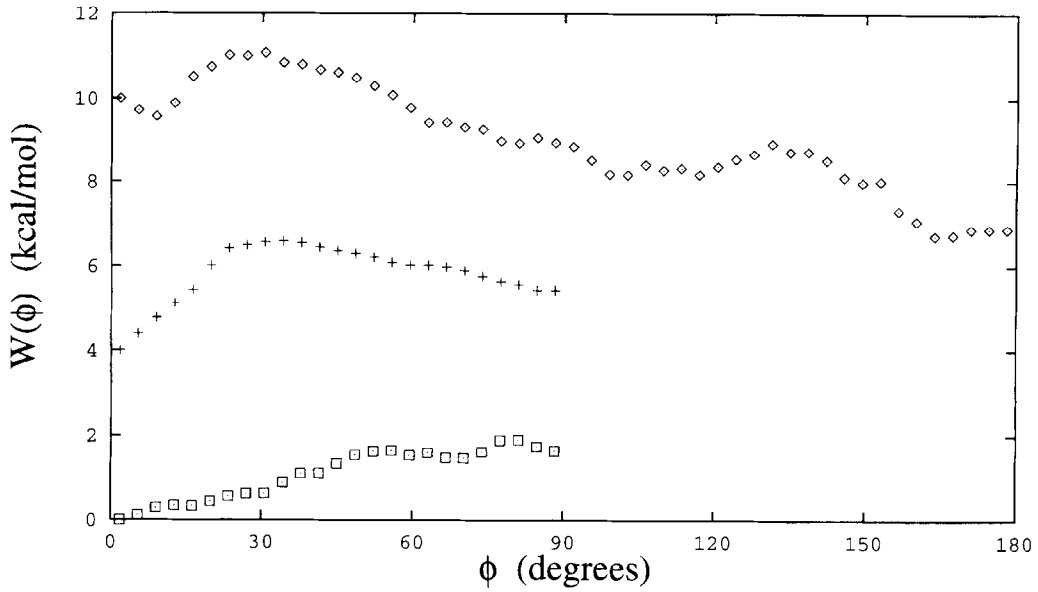

Fig. 2. Calculated potential of mean forces $W(\alpha)$ (with $\beta=\gamma=0°$) (◇), $W(\beta)$ (with $\alpha=90°$, $\gamma=0°$) (+), and $W(\gamma)$ (with $\alpha=90°$, $\beta=0°$) (□).

is also given in the column headed by E_{MC} for the conformations where the calculations have been performed.

The full calculated potential of mean forces are displayed on Fig. 2.

4. Discussion

As can be seen from Table 1 the lowest ab initio energy was obtained when all three angles took the value 0°. The AM1 calculations showed a similar trend – while the minimum is at $\alpha = 30°$ the $\alpha = \beta = \gamma = 0°$ conformation is almost as low in energy. Of the three slices of the conformational space examined, the maxima are at the same place in two of them and for most angular increments the signs of energy change are the same with both the ab initio and AM1 method.

The free energy estimates by Amsol/SM1a appear to be dominated by the electrostatic term. However, they are not correlated with the values calculated in the purely classical approach using DELPHI. In this respect it is also interesting to find that the classical electrostatic contribution to the solvation free energy is remarkably insensitive to the conformation of the molecule (the variations in the electrostatic energy is less than one kT) and as a result the correlation between the electrostatic energy and the solvation free energy is poor. The likely reason for this poor correlation is that the electrostatics is dominated by the + 1 charge on the piperazine ring and the variation of the torsion angles hardly change the general shape of the molecule. Note that this divergence between the two methods is in contrast with the earlier study cited [12]. Since in that study different molecules were compared, our results seem to indicate that the conformational FDPB approach is less sensitive to conformational changes than Amsol/SM1a.

The free energy estimates based on Amsol/SM1a and the Monte Carlo simulations are in good qualitative agreement when the AM1 calculations are used for the gas phase energies. The weaker performance of the estimate using the HF calculations for the gas phase reference is understandable and is not an indication of the relative quality of the HF or AM1 calculations since staying within the same framework of approximations is likely to result in systematic cancellation of errors. For all slices of the potential surface, the sign of the change in the free energy is the same for both the Monte Carlo and the Amsol/SM1a-AM1 estimates, but the magnitudes may differ by as much as 3.1 kcal mol^{-1}. Notice that the PMF calculations over β and γ should be compared in reference

to the $\alpha = 90°$, $\beta = \gamma = 0°$ values: at $\alpha = 90°$, $\gamma = 0°$ the free energy difference between the $\beta = 0°$ and $\beta = 90°$ states are 0.46 and 1.44 kcal mol^{-1} for the Amsol/SM1a-AM1 and the MC estimates, respectively, and at $\alpha = 90°$, $\beta = 0°$ the free energy difference between the $\gamma = 0°$ and $\gamma = 90°$ state are 2.70 and 1.64 kcal/mol for the Amsol/SM1a-AM1 and the MC estimates, respectively. Thus our results indicate that the Amsol/SM1a-AM1 difference provides a good qualitative description of the variation of the solvation free energy during conformational changes at a fraction of the cost of a free energy simulations including explicit solvents.

For the comparison of our result with the conformation optimal for binding to the DNA first note that the binding to the minor groove was found best with $\alpha = 30°$, $\beta = \gamma = 0°$. The combination of the Hartree–Fock energies and the free energy simulations predict the $\alpha = 180°$, $\beta = \gamma = 0°$. However, the Hartree–Fock contribution hardly changes in the [0°, 30°] and in the [120°, 180°] intervals, and the negative solvent contribution at 180° is nearly canceled by the Hartree–Fock energy. As a result, conformations with α in either the [0°, 30°] or the [120°, 180°] interval are thermally accessible at room temperature solution, allowing easy binding of the Hoechst agent to DNA.

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