Conformational Stability of Dimethyl Phosphate Anion in Water: Liquid-State Free Energy Simulations

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Abstract: The relative free energies of hydration of dimethyl phosphate anion in gauche–gauche, gauche–trans, and trans–trans conformations of phosphodiester torsion angles, computed with Monte Carlo method in the (T, V, N) ensemble at 25 °C, are reported. The free energy differences are determined by the probability ratio method using both umbrella sampling with harmonic weighting functions and adaptive umbrella sampling technique. The results indicate a stability trend of gg > gt > tt for the phosphodiester torsions in aqueous solutions. The magnitude of the conformational differences indicates the extended gt and tt conformations to be thermally accessible to some extent at ambient temperatures. Conclusions of the present study are in agreement with the IR and Raman inferences and ^{31}P NMR investigations. Methodologically, the performance of the free energy simulations through probability ratio method along with the adaptive umbrella sampling technique is tested on a three-legged thermocycle and found to converge in a satisfactory manner.

I. Introduction

An important set of conformational coordinates in the structural chemistry of nucleic acids is the phosphodiester torsion angles of the nucleotide backbone (α, ζ) in IUPAC notation.[1] In a previous paper from this laboratory,[2] we reported liquid-state Monte Carlo computer simulations of the mean energy of hydration (thermodynamic internal energy) of dimethyl phosphate in various phosphodiester conformations and discussed in detail structural and energetic aspects of the hydration as described by our calculations. Conformational preferences, however, are a matter of free energy rather than internal energy, and we have subsequently applied free energy simulations to this problem. Techniques for the free energy simulations are 1 order of magnitude more intensive computationally than mean energy simulations, and special methodology is involved. Specifically, we describe herein a Monte Carlo determination of the relative free energies of hydration of dimethyl phosphate anion (DMP^-) in the gauche–gauche (gg), gauche–trans (gt), and trans–trans (tt) phosphodiester conformations (Figure 1), carried out at a temperature of 25 °C and at experimental density. The relative conformational free energies here are calculated by the probability ratio method[3,4] and are compared with independent estimates of the free energy of hydration of DMP^- by the simpler hydration shell[5] and the concentric dielectric continuum[6] models for the environment. Intramolecular aspects of the problem have been described separately by the quasi-harmonic Monte Carlo method.[5]

II. Background

Conformational analysis of the nucleic acid backbone involves the six torsional angle variables α, β, γ, δ, ϵ, and ζ, where α and ζ are the phosphodiester torsion angles. The magnitude of the problem involved, in free space, is clearly depicted in Olson's[7] study based on sterically allowed rotational combinations. Each of the torsion angles α, β, γ, and ζ takes three preferred values [g^+ ($\sim$60°), t($\sim$180°), and g^- ($\sim$300°)], δ assumes two (g^+ and t), and ϵ assumes two (t and g^-), giving a total of 324 possible rotational combinations for the six torsion angles along the backbone. This, coupled with the syn and anti orientations of the bases, gives a multiplicative factor of 648 for each repeating nucleotide unit, not counting sugar pucker. Structural correlations, however, reduce the number of allowed combinations. Arnott and Hukins[8] and Sundaralingam,[9] on the basis of a statistical analysis of the known crystal structures of model compounds for nucleotides, observed that nucleotides were more rigid than nucleosides, thus forwarding the rigid-nucleotide concept. The most preferred values for α and ζ were found to be in the range of g^-, g^- for right-handed helices. The other favored conformations (g^-, t) and (g^+, t) resulted in an extended backbone structure with unstacked bases but have been conjectured to be of significance in solution and in the folding of polynucleotide chains. Berman et al.[10] proposed a categorization of the crystal structures as falling into three conformational classes: (1) minihelices, (2) extended structures, and (3) folded structures. The major distinguishing elements are the phosphodiester torsions (α and ζ), which fall into the ranges of (g^-, g^-) for class 1, (g^-, t) for class 2, and (g^+, g^+) for class 3. Thus, a condsiderable degree of conformational flexibility in oligonucleotides studied was seen to occur within the phosphodiester linkage, and thus the conformational stability as a function of these coordinates is a matter of interest.

Table I. Experimental and Theoretical Studies on Conformational Preferences of DMP^- (Energies, kcal/mol)

reference	study	gg	gt	tt
Giarda et al.[11]	crystal, X-ray	×		
Shimanouchi et al.[12]	aq soln, IR, Raman	×		
Garrigou-Lagrange et al.[13]	aq soln, DPR			x
Gorenstein[14]	aq soln, ^{31}P NMR	×		
Newton[15]	ΔU^{vac}, ab initio	0.0	3.0	7.0
Pullman et al.[16]	ΔU^{vac}, ab initio	0.0	3.4	8.0
Nanda et al.[17]	ΔU^{vac}, CNDO/2	0.0	1.5	5.0
Gorenstein et al.[18]	ΔU^{vac}, CNDO	0.0	0.1	0.9
Langlet et al.[19]	ΔU^{hyd}, Dis-Conti	0.0	−0.6	...
Alagona et al.[20]	ΔH^{hyd}, MC	0.0	28.0	...

Both theoretical and experimental studies on phosphodiester torsion angles in model compounds, in free space and in solution, were recently surveyed in our previous account of the hydration of dimethyl phosphate.[2] Results on DMP^-[11-20] are summarized

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(1) Saenger, W. *Principles of Nucleic Acid Structure*; Springer: New York, 1984.
(2) Jayaram, B.; Mezei, M.; Beveridge, D. L. *J. Comput. Chem.* **1987**, *8*, 917.
(3) Mezei, M.; Mehrotra, P. K.; Beveridge, D. L. *J. Am. Chem. Soc.* **1985**, *107*, 2239.
(4) Mezei, M. *J. Comput. Phys.*, in press.
(5) Jayaram, B.; Ravishanker, G.; Beveridge, D. L. *J. Phys. Chem.*, in press.
(6) Jayaram, B.; Beveridge, D. L. *Biopolymers*, in press.
(7) Olson, W. *Topics in Nucleic Acid Structure*, 2nd ed.; Neidle, S., Ed.; Macmillan: London, 1981.
(8) Arnott, S.; Hukins, D. W. L. *Nature (London)* **1969**, *224*, 886.
(9) Sundaralingam, M. *Biopolymers* **1969**, *7*, 821.
(10) Berman, H. M.; Shieh, H.-S. *Topics in Nucleic Acid Stucture*, 1st ed.; Neidle, S., Ed.; Macmillan: London, 1981.
(11) Giarda, L.; Garbassi, F.; Calcaterra, M. *Acta Crystallogr., Sect. B: Struct. Crystallogr. Cryst. Chem.* **1973**, *B29*, 1826.
(12) Shimanouchi, T.; Tsuboi, M.; Kyogoku, Y. *Adv. Chem. Phys.* **1964**, *7*, 435.

0002-7863/88/1510-1691$01.50/0

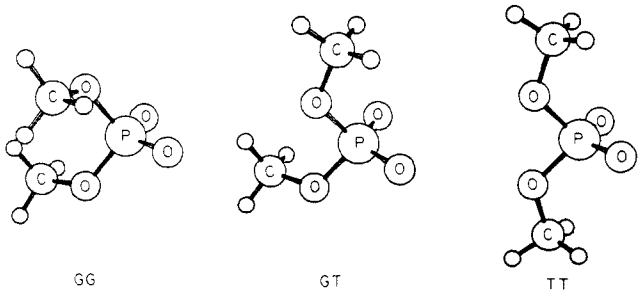

Figure 1. Molecular structure of dimethyl phosphate anion in gauche–gauche (gg), gauche–trans (gt), and trans–trans (tt) conformations.

in Table I. All earlier theoretical investigations are focused on the internal energy contributions to conformational differences. There is no information so far on the entropic contributions to the conformational free energies both in free space and in aqueous solutions. In a study carried out in parallel with this project, we used quasi-harmonic Monte Carlo methods to estimate the intramolecular thermodynamics of dimethyl phosphate anion in free space.[5] The computed conformational free energies relative to gg were 0.7 kcal/mol for gt and 1.6 kcal/mol for tt conformation. Here, in the following, we describe the evaluation of relative conformational free energies of hydration through liquid-state Monte Carlo computer simulations.

Liquid-state computer simulations and Monte Carlo method in particular have proven to be successful in evaluating structural and thermodynamic quantities such as radial distribution functions and internal energies. Thermodynamic quantities such as free energy are not straightforward to obtain from simulations since the Monte Carlo and molecular dynamics techniques avoid computing partition functions due to convergence problems. Efforts in this direction have been extensively reviewed in a recent paper from this laboratory.[21] Higher energy regions of the configurational space, extremely important for the estimation of free energy, are either undersampled or not sampled at all in any scheme designed simply to cull configurations with high Boltzmann probabilities. Non-Boltzmann sampling[22] (aimed at extracting ultimately Boltzmann averages, of course) is a way to circumvent the above mentioned problem. A coupling parameter approach[3] was developed in this laboratory that, along with the umbrella sampling,[22] permits estimates of the relative free energies via probability ratios. This method was recently applied successfully to estimate the relative free energies of hydration of the conformations of alanine dipeptide in water.[3] The probability ratio method was subsequently extended to incorporate a self-consistent determination of the non-Boltzmann bias,[4] by a procedure called "adaptive umbrella sampling".

Computer simulations on ionic systems are particularly problematic due to the strong and long-ranged nature of the solute–water interactions. Free energy studies on ionic systems reported so far are relatively few and mostly confined to simple systems. Thus, the practical applicability of the different free energy methodologies[21] to multifunctional solutes and in particular to biopolymers and their limitations such as the extent of uncertainties on the quantitative estimates merit further investigation. An assessment of the probability ratio method, its application to aqueous solutions of nucleic acid constituents, and specifically evaluation of relative conformational free energies of hydration of phosphodiester torsions in DMP^- are presented in the following. Special attention was given to the performance of the simulation methodology on the thermodynamic cycle.

III. Theory and Methodology

To estimate the hydration free energy difference between any two conformations i and j, ΔA_{ij}^{hyd} by computer simulations, a coupling parameter (λ) is introduced to accomplish a smooth transition of the system from one state ($\lambda = 0$) to another ($\lambda = 1$) during the calculation. Thus any intermediate conformation $\{q_\lambda\}$, between conformations $\{q_i\}$ and $\{q_j\}$, along the one-dimensional path defined by the coupling parameter, is generated as eq 1, where

$$\{q_\lambda\} = \lambda\{q_i\} + (1 - \lambda)\{q_j\} \quad (1)$$

λ is identifiable with a correlated conformational coordinate[21] and $\{q_i\}$ denotes the atomic coordinates of the molecule in conformation i. A similar equation holds for the partial atomic charges. The free energy of hydration can be expressed as

$$A^{hyd}(\lambda) = -k_B T \ln P(\lambda) + \text{constant} \quad (2)$$

where $P(\lambda)$ is the unnormalized probabilities of occurrence of the state corresponding to the value of the coupling parameter λ. The problem then is reduced to developing $P(\lambda)$ where $\lambda \in [0, 1]$. A sequence of Monte Carlo runs with umbrella sampling with an appropriate weighting function produces probabilities of state i and j and all other states along the path defined by the coupling parameters. Whereas in the umbrella sampling technique the weighting function is conventionally chosen as a harmonic function of the form

$$E_w(\lambda) = c(\lambda - \lambda_0)^2 \quad (3)$$

the adaptive umbrella sampling scheme is based on the logic that the best weighting function is the free energy function itself.

$$E_w(\lambda) = k_B T \ln P(\lambda) \quad (4)$$

Since $E_w(\lambda)$ is not known a priori, an iterative approach is used where $P(\lambda)$ is first estimated on a smaller set of λ with the weighting function set to zero. E_w is then obtained by eq 4, and this estimate is used in the next step to enlarge the set of λ sampled. This refinement is continued until the adequate $E_w(\lambda)$ is found.

A ratio of the unbiased probabilities, properly matched, then yields relative free energies of hydration between any two states defined by the coupling parameter. The probabilities for the whole range of [0, 1] of λ in the present study are matched at the values of λ where the slopes of successive $[-\ln P(\lambda)]$ curves are equal. Finally, the relative free energy of hydration between the conformations i and j is obtained as

$$\Delta A_{ij}^{hyd} = A_j(\lambda = 1) - A_i(\lambda = 0) = k_B T \ln [P(\lambda = 0)/P(\lambda = 1)] \quad (5)$$

IV. Calculations

Monte Carlo computer simulations in the (T, V, N) ensemble at a temperature of 25 °C were performed to estimate relative free energies of hydration between (1) gg and tt and (2) gg and gt conformations of DMP^- in water. All characteristics of the calculations conform as closely as possible to our previously reported mean energy simulations.[2] A modified Metropolis procedure[23] incorporating force bias[24] and preferential sampling[25] was used. The geometries chosen for the solutes were those of Gorenstein et al.[26] Partial atomic charges were computed with the GAUSSIAN-80 system of programs[27] with the atomic orbital basis sets

(13) Garrigou-Lagrange, C.; Bouloussa, O.; Clement, C. *Can. J. Spectrosc.* **1976**, *21*, 75.
(14) Gorenstein, D. G. In *Phosphorous-31 NMR, Principles and Applications*; Gorenstein, D. G., Ed.; Academic: New York, 1984; Chapter 1.
(15) Newton, M. D. *J. Am. Chem. Soc.* **1973**, *95*, 256.
(16) Pullman, B.; Gresh, N.; Berthod, H. *Theor. Chim. Acta* **1975**, *40*, 71.
(17) Nanda, R. K.; Govil, G. *Theor. Chim. Acta* **1975**, *38*, 71.
(18) Gorenstein, D. G.; Luxon, B. A.; Findlay, J. B. *Biochim. Biophys. Acta* **1977**, *475*, 184.
(19) Langlet, J.; Claverie, P.; Pullman, B.; Piazzola, D. *Int. J. Quantum Chem. Biol. Symp.* **1979**, *6*, 409.
(20) Alagona, G.; Ghio, C.; Kollman, P. A. *J. Am. Chem. Soc.* **1985**, *107*, 2229.
(21) Mezei, M.; Beveridge, D. L. "Free Energy Simulations", in *Computer Simulations and Biomolecular Systems*; Beveridge, D. L., Jorgensen, W. L., Eds.; Annals of the New York Academy of Sciences 494; New York Academy of Sciences: New York, 1986.
(22) Valleau, J. P.; Torrie, G. M. *Modern Theoretical Chemistry*; Berne, B. J., Ed.; Plenum: New York, 1977; Vol. 6, Chapter 5.
(23) Metropolis, N.; Rosenbluth, A. W.; Rosenbluth, M. N.; Teller, A. H.; Teller, E. *J. Chem. Phys.* **1953**, *21*, 1087.
(24) Rao, M.; Pangali, C.; Berne, B. J. *Mol. Phys.* **1979**, *37*, 1773.
(25) Owicki, J. C.; Scheraga, H. A. *Chem. Phys. Lett.* **1979**, *47*, 600.
(26) Gorenstein, D. G.; Luxon, B. A.; Findlay, J. B. *Biochim. Biophys. Acta* **1977**, *475*, 184.
(27) GASSIAN-80, QCPE, adapted for IBM by S. Topiol and R. Osman.

of Matsuoka et al.[28] and correspond to the tt form. The system in each case consisted of DMP^- and 215 water molecules at experimental density. FCC (face-centered cubic) periodic boundary conditions were used to simulate the macroscopic system. The intersolute interactions were neglected; thus, the system corresponds to one at infinite dilution. Solute–water interactions were computed with the potential functions of Clementi et al.,[29] under minimum image convention. Water–water interactions were modeled by the MCY representation,[30] with a spherical cutoff of 7.75 Å. In the set gg to tt, the coupling parameter λ carried the phosphodiester torsions from (60°, 60°) to (180°, 180°) and the O–P–O valence angle from 103.4° to 92°, in accordance with eq 1. Similarly, in the set gg to gt, the torsion angles were varied from (60°, 60°) to (60°, 180°) and the O–P–O valence angle from 103.4° to 97.5°, along with the partial atomic charges.

Preliminary calculations with harmonic weighting functions for the non-Boltzmann bias involved a total of six Monte Carlo simulations for gg to tt and five for gg to gt. Convergence of these runs was established by monitoring the control function defined on the internal energy of hydration as described in ref 31. The results suggested that, in hydration, gg was more stable than tt by 6.4 ± 1.0 kcal/mol, and gt, by 7.2 ± 1.0 kcal/mol. The stability of the gg conformer relative to gt and tt predicted by these simulations was consistent with the observed prevalence of the gg form in crystal studies of oligonucleotides. As a self-consistency check on the methodology and its quantitative predictions, six more Monte Carlo runs were conducted from gt to tt in an attempt to close the thermocycle. As against a value of 0.8 kcal/mol in favor of the tt conformer to be expected from the previous two sets, the computed value for the free energy of hydration of gt relative to tt was −12.2 kcal/mol. While this suggested an ordering of gg > gt > tt for the relative preferences of phosphodiester torsions in DMP^- in water, the free energy cycle was off by 13 kcal! This raised serious questions about convergence of the Monte Carlo runs, the statistical noise in the estimated probabilities, the errors involved in matching, and the path dependence of the computed quantities. Convergence of a macroscopic quantity may not ensure the closure of the thermocycle. A microscopic and more rigorous convergence criterion was then defined on the path itself. A control function was defined on the coupling parameter, and its behavior was monitored to ensure that the values of the coupling parameter fluctuated randomly about a given mean value in each Monte Carlo run. This scheme provides a way of ensuring that the umbrella sampling scheme with harmonic weighting functions is properly implemented. A total of 20 more Monte Carlo simulations were performed meeting this criterion. The stability trend for the phosphodiester torsions in DMP^- in water was again observed to be gg > gt > tt as above. The first set from gg to tt gave a free energy of hydration of 6.1 ± 0.6 kcal/mol in favor of gg. This was checked by a second set from tt to gg, which yielded −5.6 ± 0.6 kcal/mol indicating that each segment of the cycle is reproducible to within 1 kcal/mol. The thermocycle (gg → gt → tt → gg), however, was still off by 8 kcal. While there is no obvious theoretical explanation for this anomalous result, the rotation of the solute during the simulations, particularly when the solute–water interactions arising in waters external to the first shell make significant contribution to solute binding energies, may lead to a very slow convergence of the estimated probabilities, since the "corners" of the simulation cell provide a slightly anisotropic environment. The influence of the selected harmonic weighting function and that of the initial configuration on the numerical estimates are observed to be nonvanishing in runs of length less than 1000K.

The hydration free energy calculations were repeated on the gg, gt, and tt conformations of DMP^- with the probability ratio method by adaptive umbrella sampling technique involving a self-consistent determination of the non-Boltzmann bias.[4] Furthermore, solute was held rotationally fixed. Each of the two calculations (gg to tt and gg to gt) involved a total of three simulations, each of run length 2000K Monte Carlo steps. The performance of these simulations now appears to be satisfactory, with the thermocycle closing within a fraction of kilocalories/mole. Results of these calculations are presented in detail in the following section. The corresponding matching curves are given in Figure 2. Calculations on a third set from gt to tt showed a double maxima for the $-kT \ln P(\lambda)$ curve and required five Monte Carlo simulations. This set together with the two above indicating that the statistical noise for the complete thermocycle simulation is ~0.6 kcal, which we feel is reasonable considering that a total of 11 simulations were involved in all.

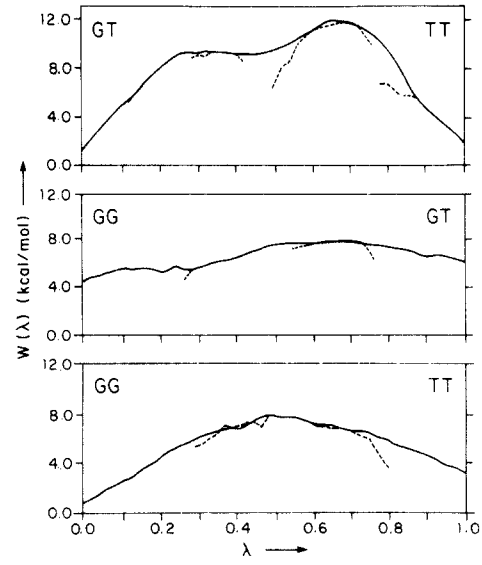

Figure 2. $-kT \ln P(\lambda)$ versus λ curves for gg to tt (bottom), gg to gt (middle), and gt to tt (top) free energy simulations with adaptive umbrella sampling technique.

Table II. Energetics of Hydration of DMP^- (kcal/mol)

	gg	gt	tt
ΔA^{hyd} [a]	0.0	1.9 ± 0.1	2.3 ± 0.5
ΔU^{hyd} [b]	0.0	−5.3 ± 3.5	4.7 ± 2.3
$T\Delta S^{hyd}$	0.0	−7.2 ± 3.5	2.4 ± 2.4
ΔA^{hyd} (HSM)[c]	0.0	0.4	1.8
ΔA^{hyd} (CDC)[d]	0.0	2.6	3.3

[a] The calculated relative free energies of hydration discussed in the text. [b] The mean internal energies of hydration taken from ref 2. [c] Relative free energies of hydration estimated through hydration shell model.[5] [d] Relative free energies of hydration evaluated through concentric dielectric continuum model.[6]

V. Results and Discussion

The calculated free energies of hydration are collected in Table II. The reported uncertainties in the estimated free energies reflect errors deduced from the differences in probability estimates of overlapping segments (i.e. errors incurred in matching). The stability ordering (row 1) for the phosphodiester torsions of DMP^- in water as suggested by these computations is gg > gt > tt. This is consistent with the observed prevalence of the gg conformer and lack of evidence for tt form in oligonucleotides. Shimanouchi et al.[12] interpreted the IR spectrum of $Ba^{2+}(DMP^-)_2$ and the Raman spectrum of Na^+DMP^- in aqueous solution by means of normal-coordinate calculations and concluded that the gg form was the likely conformation for DMP^- in both solid and solution. Gorenstein and co-workers[14] investigated the ^{31}P chemical shifts of DMP^- and related compounds in aqueous solution as a function of temperature in conjunction with studies of nucleic acid conformation. The ^{31}P resonance shifts downfield with increasing temperature. This was interpreted by an increase in the Boltzmann population of gt and possibly tt conformers. By inference, the preponderance of gg conformation of DMP^- at lower temperatures, including ambient, was indicated. The preference for the gg conformer of phosphodiester torsions in aqueous solutions inferred in the above spectroscopic studies is in concordance with the results of the present calculations. Table II gives a split of ΔA^{hyd} (row 1) into ΔU^{hyd} (row 2) and $T\Delta S^{hyd}$ (row 3) contributions. ΔU^{hyd} values are taken from the Monte Carlo mean energy simulations reported earlier.[2] The results show that while internal energies of hydration favor gt conformer, entropically the gt form turned out to be least favored. The calculated entropies are subject to considerable statistical uncertainty, however.

The stability of gg conformation for the phosphodiester torsion angles over the extended forms in free space has previously been attributed to anomeric effects.[1] Our previous mean energy simulations on the aqueous solutions of DMP^-[2] showed that ionic

(28) Matsuoka, O.; Tosi, C.; Clementi, E. *Biopolymers* **1978**, *17*, 33.
(29) Clementi, E.; Corongiu, G.; Lelj, F. *J. Chem. Phys.* **1979**, *70*, 3726.
(30) Matsuoka, O.; Clementi, E.; Yoshimine, M. *J. Chem. Phys.* **1980**, *72*, 3979.
(31) Mezei, M.; Swaminathan, S.; Beveridge, D. L. *J. Chem. Phys.* **1979**, *71*, 3366.

hydration destabilizes the tt conformation relative to both gg and gt, while gt was favored over gg conformer. The hydration free energy simulations reported here indicate that the relative preference of the phosphodiester torsions for gg conformation over gt form in aqueous solutions is to be traced to the $T\Delta S$ term. This is an interesting result and is in accord with the current view of the hydrophobic effect, given that the two methyl groups are closer to each other in the gg conformation. This result also supports the inferences of Jorgensen[32] on conformational evidence for the hydrophobic effect. In his Monte Carlo simulations of *n*-butane in water, the gauche conformer was found to be preferentially solvated. The calculations here establish the relevance of hydrophobic effect to the conformational preferences of biomolecules in aqueous solutions.

Also shown in Table II in the last two rows are the relative free energies of hydration estimated through hydration shell model[5] (row 4) and concentric dielectric continuum calculations[6] (row 5). The conformational preferences predicted by these two models are in agreement with the trends obtained through the free energy simulations here (row 1). The continuum description of hydration appears to overestimate the conformational differences compared with the results obtained from the liquid-state free energy simulations. Continuum representation of the solvent underscores contributions arising solely from the attractive part of the potential of mean force and neglects the nonelectrostatic contributions such as due to shape, size, and packing configurations of the solvent molecules. By way of contrast, the hydration shell model emphasizes the local structural factors and appears to underestimate the free energy differences. Simulations take both these aspects into consideration. The overall picture presented by the conformational differences evaluated through simulations here is that the calculated hydration free energy surface is relatively flat.

The present study yields information for the first time on the relative conformational free energies of hydration of phosphodiester torsions in nucleic acid constituents and also highlights some of the problems to be encountered in complex systems. The series of computations here have shown that qualitative trends in relative hydration free energies evaluated through simulations are reproducible with consistency. Quantitative estimates, however, are more exacting. Thus, caution is to be exercised in drawing inferences based on the assumption that the numerical estimates of hydration free energies of larger systems evaluated through free energy simulations honor the thermodynamic cycle. Thermocycle provides a stringent test on the methodology, and an error estimate of 0.6 kcal obtained here is very encouraging.

Subsequent studies are being undertaken to clarify the sensitivity of results to the assumed change distribution.

VI. Conclusions

The free energy simulations reported here indicate that hydration stabilizes the gg conformation for the phosphodiester torsions in DMP^-. The anionic hydration through the solute–solvent interactions and the hydrophobic hydration through the entropic contributions are observed to be the major factors stabilizing the gg conformer of DMP^- in aqueous solutions. The conformational differences in free energies of hydration however suggested that the extended forms (gt and tt) may be thermally accessible to some extent in aqueous solutions at ambient temperature. On the methodological front, the performance of the free energy simulations is satisfactory.

Acknowledgment. This research was supported by NIH Grant GM 24914 and by NSF Grant CHE-8203501.

Registry No. DMP^-, 813-78-5.

(32) Jorgensen, W. L. *J. Chem. Phys.* **1982**, *77*, 5757.

Molecular and Electronic Structure of the Low-Lying Electronic States of Cycloalkenylidenes: Cyclopropenylidene

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Abstract: Results of semiempirical (MNDO) and ab initio (at the SCF, TCSCF, and CISD levels of theory) calculations on the low-lying electronic states of cyclopropenylidene are reported. In addition to the ground state, which is predicted to be the closed-shell singlet $^1A_1$, three open-shell singlets ($^1A_2$, $^1B_1$, $^1B_2$), two triplets ($^3A_2$ and $^3B_1$), and one doubly excited closed-shell singlet (2^1A_1) are described. It is found that at the ab initio SCF level of theory the open-shell states of A_2 symmetry exhibit three imaginary frequencies due to the fact that the wave function of these states is subject to Hartree–Fock instability. As expected, the electron charge distribution, and therefore the electrophilic or nucleophilic character of the carbene center, changes significantly along the seven electronic states considered. However, owing to the large energy differences between the ground state and the calculated excited states, it is unlikely that the latter may play any significant role in the chemistry of (singlet) cyclopropenylidene.

I. Introduction

The chemistry of carbenes is normally interpreted in terms of the relative energies of the lowest singlet (S_0) and triplet (T_1) electronic states of these species.[2] In methylene, the simplest carbene, the above states arise from occupation with two electrons of the lone-pair σ-orbital (which is basically a sp^2 hybrid orbital in the molecular plane) and the out-of-plane p atomic orbital (AO) on the carbon atom. Essentially, the singlet S_0 is fairly well described by the configuration σ^2p^0 whereas the triplet T_1 by the configuration σ^1p^1. In addition to these low-lying states, there are two possible excited singlet states, S_1 and S_2, arising from configurations σ^1p^1 and σ^0p^2, respectively. Both ab initio and semiempirical molecular orbital (MO) calculations[3] indicate that

(1) (a) Department de Química Orgànica. (b) Departament de Química Física.

(2) Kirmse, W. *Carbene Chemistry*; Academic: New York, 1971. Gaspar, P. P.; Hammond, G. S. In *Carbenes*; Moss, R. A., Jones, M., Eds.; Wiley: New York, 1975; Vol. 2. Moss, R. A.; Jones, M. In *Reactive Intermediates*; Jones, M., Moss, R. A., Eds.; Wiley: New York, 1981; Vol. 2.

(3) For a recent review on theoretical calculations of methylene, see: Shavitt, I. *Tetrahedron* **1985**, *41*, 1531.

0002-7863/88/1510-1694$01.50/0