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Free energy simulations: Applications to the study of liquid water, hydrophobic interactions and solvent effects on conformational stability

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Abstract. Free energy simulations using the Metropolis Monte Carlo method and the coupling parameter approach with umbrella sampling are described for several problems of interest in structural biochemistry; the liquid water, the hydrophobic interaction of alkyl and phenyl groups in water and solvent effects on the conformational stability of the alanine dipeptide and the dimethyl phosphate anion in water. Proximity analysis of results is employed to identify stabilizing factors. Implications of result with respect to the structural chemistry of proteins and nucleic acids is considered.

Keywords. Free energy; simulations; hydrophobic interactions; solvent effects in conformation.

Introduction

With the present generation of digital computers and now supercomputers, systems of molecules representative of the liquid state have become accessible to study by large scale numerical methods based on statistical thermodynamic and molecular dynamic theories of mixtures, and referred to as "computer simulations". Recent years have seen the emergence of computer simulation studies on liquid water (Beveridge *et al.,* 1983), aqueous solutions (Beveridge *et al.,* 1981), and the initial extensions of these procedures to the study of biomolecules in solution (Clementi, 1980; Corongiu and Clementi, 1981; Finney *et al.,* 1985). In principle, calculation of many useful thermodynamic variables and molecular indices of hydration can be obtained from computer simulations on aqueous solutions. However, research methodologies in this field are still very much under development, and much remains to be learned both about and from computer simulation applied to biomolecular systems. One of the most interesting and urgent areas of current study in computer simulation is the determination of free energy.

Statistical thermodynamics tells us that the basic relationship between the configureational energy and the excess free energy A of a system is *via* the partition function Z,

$$A = -kT\,ln\,Z/V^N = -kT\,ln\,\langle \exp\,(+E/kT)\rangle/V^N \tag{1}$$

where k is the Boltzmann's constant, T is temperature and the brackets denote a Boltzmann configurational average or expectation value of the enclosed quantity. For the free energy difference between two different systems with potentials E_o and E_1, eq. (1) gives

$$\Delta A = -kT \ln \langle \exp [-(E_1 - E_0)/kT] \rangle \quad (2)$$

Conventional Monte Carlo and molecular dynamic computer simulations although firmly grounded in Boltzmann statistical mechanics and dynamics do not involve the computation of a partition function due to convergence problems. The Metropolis method (Metropolis *et al.,* 1953) was in fact cleverly designed to avoid this difficulty in the calculation of mean energy by Markov-chain Monte Carlo simulation. In molecular dynamics, the physical nature of the calculated particle trajectories serves this purpose at least equally well. However, in the absence of a partition function one is unable to compute the free energy directly in a conventional Monte Carlo or molecular dynamics calculation. The determination of the free energy *via* the expectation value approach using eq. (2) encounters difficulties of similar origin except when S_0 and S_1 are quite similar (Sarkisov *et al.,* 1974; Owicki and Scheraga, 1977). Thus under ordinary circumstances we lack access in computer simulation to free energy, the fundamental index of thermodynamic stability of the system.

A number of approaches to this problem have been suggested. For several years now we have been working with the "coupling parameter approach" which involves a parameter that carries the system smoothly from a reference state $\xi = 0$ to the system of interest $\xi = 1$. In a modern sense this approach originates in the derivation of an important integral equation in liquid state theory by Kirkwood (1968). The coupling parameter approach used in conjunction with the procedures of umbrella sampling developed more recently by Valleau and coworkers (Patey and Valleau, 1975; Torrie and Valleau, 1977) provides a means of calculation of free energy in computer simulations applicable to diverse problems in structural biochemistry. In this account we describe briefly the coupling parameter approach to free energy simulations, and review recent free energy simulation studies carried out in this laboratory on liquid water, prototype systems for the study of hydrophobic interactions, and problems in the area of solvent effects on conformational stability of protein and nucleic acid constituents.

Theory

In conventional mean energy Monte Carlo or molecular dynamics computer simulations, one treats a system of particles such as the atoms of a protein, the molecules of a liquid, and most recently, both together (Berendsen, 1984). In condensed phase problems, the system is usually configured in a cell subject to periodic boundary conditions. The configurational energy of individual complexions of the system and configurational forces on individual molecules are evaluated from analytical energy functions parameterized from experimental data or quantum mechanical calculations. From this point on, computer simulation on molecular systems is fundamentally a numerical integration of the mean energy expression and other ensemble averages on the computer, with the sampling carried out with a probabilistic (Monte Carlo) or deterministic (molecular dynamics) strategy. In a typical study, structural, energetic and dynamical properties of the system are often of greater interest than mean energy, but the stability of this quantity remains the basic index of convergence in the calculations.

The quality of the mean energy is directly dependent upon the quality of intermolecular potentials used in the simulation.

The extension of mean energy simulation to the determination of free energy is best described by considering two states of a chemical system, S_0 and S_1, which may differ from each other in any conceivable way. Let us define a free energy function A(ξ) where ξ is a coupling parameter which on the interval from 0 to 1 carries the reference S_0 into the system of interest S_1 The free energy difference between S_0 and S_1 is then

$$\Delta A = \int_0^1 \langle \frac{\partial}{\partial \xi} E(\xi) \rangle_{E(\xi)} \mathrm{d}\xi \tag{3}$$

where the subscript E (ξ) denotes a Boltzmann average on the energy function E (ξ). The discussion here is carried out based on the Helmholtz free energy and the (T, V, N) ensemble but can be easily extended to Gibbs free energy and the (T, P, N) ensemble. The simplest applications of the coupling parameter approach, with ξ identified with the volume or the inverse temperature of the system, lead to the virial expression for pressure and the van't Hoff equation respectively, textbook cases in physical chemistry.

The applications of the coupling parameter approach of interest in our current computer simulation studies involve topographical changes in the system. We refer this to the molecular landscape in the problem under consideration. There are three broad classes of topographical transition coordinates: structural transition coordinates, reaction coordinates, and creation/annihilation coordinates as summarized in figure 1. The use of the coupling parameter approach on each of these coordinates is illustrated in the applications described below.

Alternative approaches to the free energy problem include simulations in the (T, V, μ) ensemble (Adams, 1975; Norman and Filinov, 1969), the particle insertion method (Windom, 1963; Romano and Singer, 1979; Shing and Gubbins, 1981, 1982; Powles *et al.,* 1982) and overlap ratios (Jacucci and Quirke, 1982; Quirke and Jacucci, 1982).

Liquid water studies

For the special case of E (ξ) linear in ξ,

$$E(\xi) = (1-\xi)E_0 + \xi E_1, \tag{4}$$

$$\Delta A = \int_0^1 \langle E_1 - E_0 \rangle_{E(\xi)} \mathrm{d}\xi. \tag{5}$$

Numerical realizations of eq. (5) involve a series of simulations, carried out developing mean energy as a discrete function of coupling parameter, with the final integration over ξ carried out numerically.

Several calculations of free energy *via* eq. (5), or the equivalent are now to be found in the literature (Mruzik *et al.,* 1976; Berens *et al.,* 1983; Swope *et al.,* 1982) and are reviewed (Beveridge and Mezei, 1985). In this laboratory Mezei *et al.* (1978) carried out a determination of the free energy of liquid water using this approach. Monte Carlo simulations were carried out on a system of 64 water molecules under periodic boundary conditions at a density of 1 gm/cm^3 and a temperature of 25°C. The reference state for this calculation was chosen as ideal gas, so E_0 is zero and eq. (4) reduces to

$$E(\xi) = \xi E_1, \tag{6}$$

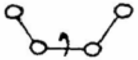
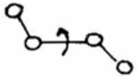
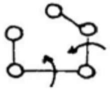
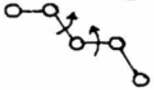
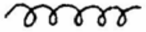
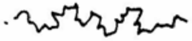
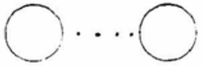
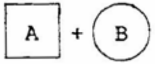
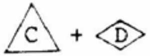
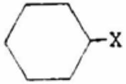
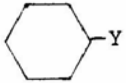
Type		$\xi = 0$	$\xi = 1$	Example
A.1	Conformational transition coordinate			g⟷t in butane, cis⟷trans in N-methyl acetamide.
A.2	Correlated conformational transition coordinate			$C_7 \rightarrow \alpha_R$ in Ala dipeptide, gg⟷gt⟷tt interconversior in dimethyl phosphate.
A.3	Structural transition coordinate			Protein denaturation, A,B,...,Z interconversions innucleic acids.
B.1	Simple reaction coordinate			Hydrophobic interactions, hydrogen binding, ion pairing.
B.2	Complex reaction coordinate			Organic SN2 reaction, $Cl^- + CH_3Cl \rightarrow CH_3Cl + Cl^-$
C.1	Functional group (or residue) creation/ annihilation coordinate			Homologous series of substrates, inhibitors or agonists.
C.2	Molecule creation annihilation coordinate			Hydration potential of diverse biological molecules.

Figure 1. Examples for the various applications of the coupling parameter.

where E_1 is the configurational energy of the assembly of water molecules. The parameter ξ in this application couples in the intermolecular interactions, and ξ may thus be considered a creation/annihilation coordinate where only the creation branch is active. In the context of thermodynamic integration, ξ can be considered as a temperature weighting factor and the ξ coordinate a trans-critical tieline.

The calculated $\langle E \rangle_{E(\xi)}$, *Edenoted* U *(ξ),* for liquid water is shown in figure 2. The free energy was determined by an 8-point Gaussian quadrature over ξ. The calculated free energy turned out to be – 4·31 ± 0·7 kcal/mol *vs.* an observed value of – 5·74 kcal/mol. The discrepancy is due primarily to deficiencies in the intermolecular potential function (Beveridge *et al.,* 1983).

More recently, Mezei described calculations of the relative free energy of three different water models; MCY, ST2 and SPC, using the coupling parameter approach (Mezei, 1982). A soft sphere reference state was established for calculation of the free energy of the MCY water and then MCY water was used as a reference state for the ST2 and SPC determination. Here the coupling carries the liquid water smoothly from one model to another, an application of ξ as a creation/annihilation coordinate. Mezei also explored the umbrella sampling method developed by Valleau, Patey and Torrie

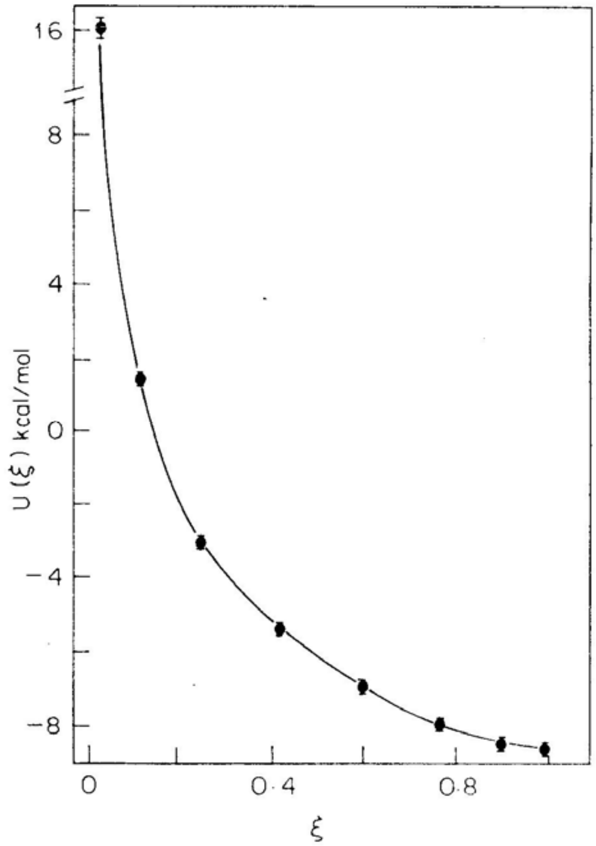


Figure 2. **U(ξ)** as a function of the coupling parameter for the computation of the free energy of the MCY water Mezei *et al.* (1978).

(Patey and Valleau, 1975; Torrie and Valleau, 1977) in conjunction with eq. (2) and found it unsuitable for this problem since the two states S_0 and S_1 were too different. This suggested the use of coupling parameter in combination with umbrella sampling to achieve a multi-stage umbrella sampling where the similitude condition is established by a succession of intermediate states. However, even when three stages were tried, no consistent results could be obtained. Several other applications of multi-stage sampling and variations are found in the current literature (Torrie and Valleau, 1977; Miyazaki *et al.,* 1976; Scott and Lee, 1980).

Hydrophobic interactions

A different line of approach to free energy simulations involing the coupling parameter, particularly useful but not restricted to comformational changes, is to determine A (ξ). However, the ξ-coordinate usually cannot be considered as simply another degree of freedom in an otherwise conventional mean energy simulation. In this case the realization of the system, seeking to describe the equilibrium state dictated by the Boltzmann equation, would end up sampling only a narrow range of ξ rather than the full interval from 0 to 1. Thus sampling of ξ requires special, non-Boltzmann sampling techniques beyond those typically used in mean energy simulations.

The current way to proceed on potential of mean force calculations is to apply the umbrella sampling method. In this approach a series of simulations are carried out, each constrained to sample the local region about points ξ_1, ξ_2, ξ_3 . . ., respectively on the interval from 0 to 1. In an individual simulation, a distribution function g (ξ) is obtained. A particular ξ-simulation is constrained to sample a region about ξ by adding a harmonic constraint to the configurational energy, *viz*

$$V(\xi) = E(\xi) + E_H(\xi), \tag{7}$$

$$E_H(\xi) = k_H(\xi - \xi_0)^2, \tag{8}$$

as first employed in this form by Pangali *et al.* (1979a, b). The non-Boltzmann bias in the results due to the presence of the harmonic term can be removed by means of Valleau's equation,

$$g(\xi') = \langle \delta(\xi' - \xi) \exp[E_H(\xi)/kT] \rangle_{V(\xi)} / \langle \exp[E_H(\xi)/kT] \rangle_{V(\xi)} \tag{9}$$

where δ ($\xi' - \xi$) is the Dirac delta counting function for configurations with coupling parameter ξ. Successive points are chosen on ξ such that g (ξ) are overlapping. Overlapping points in the distribution correspond in principle to the same absolute value but in practice differ by a normalization constant. Thus the various computed g (ξ) can be shifted up or down, and ultimately matched up to produce a g (ξ) for the entire range of ξ The matching can in principle be carried out for any overlapping points, but in practice one chooses those points with relatively low statistical noise levels. An alternative procedure where the harmonic potential E_H is replaced by a tabulated function determined from the simulation itself, is currently under testing (Mezei, M., unpublished results). This biasing potential appears to be capable to cover a much larger range of the coupling parameter than E_H. Once g (ξ) has been obtained, the corresponding free energy A (ξ) can be evaluated as

$$A(\xi) = -kT \ln g(\xi) + C, \tag{10}$$

known as the potential of mean force, commonly denoted *W*(*R*). Details of the methodology are described further by Pangali *et al.* (1979a,b). Several potential of mean force determinations using computer simulation are now to be found in the recent literature (Pangali *et al.,* 1979a, b; Ravishanker *et al.,* 1982; Berkovitz *et al.,* 1984). Umbrella sampling is also used in conjunction with eq. (2) making it viable for calculations between less similar systems (Torrie and Valleau, 1977; Owicki and Scheraga, 1978; Miyazaki *et al.,* 1976; Scott and Lee, 1980; Postma *et al.,* 1982). A notable free energy simulation has recently been reported by Jorgensen and his group who determined the potential of mean force on the complex reaction coordinate of the organic S_N^2 reaction of CH_3C1 and Cl^- in water (Chandrasekhar *et al.,* 1984) and DMF (Chandrasekhar *et al.,* 1985). The reaction is predicted to be concerted in water but to proceed *via* a reaction intermediate in DMF, a previously unanticipated result.

Potential of mean force calculations from this laboratory have been directed to the study of the interactions of apolar molecules in water, prototype systems for the theoretical study of the hydrophobic effect. Ravishanker, Mezei and Beveridge reported a potential of mean force for the interaction of two methane molecules in water obtained from Monte Carlo computer simulation (Ravishanker *et al.,* 1982). The behavior of *W* (*R*) as a function of intersolute coordinate, as shown in figure 3, turned out to be oscillatory, with successive free energy minima corresponding to contact and solvent-separated hydrophobic interactions. This intriguing result, anticipated by F. Franks (Clark *et al.,* 1977; Franks, 1975,1977; Franks and Ives, 1966) and noted in

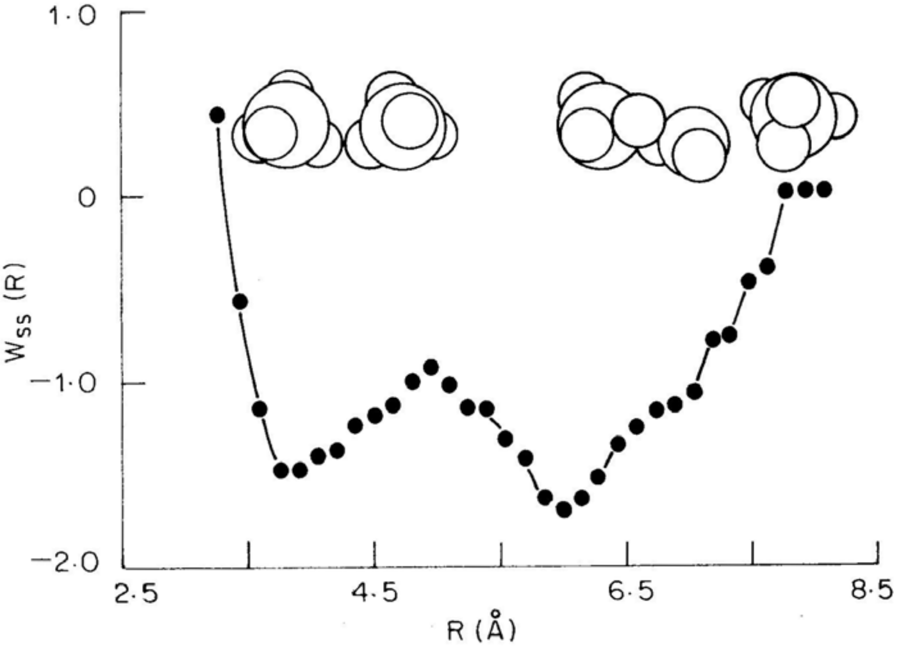


Figure 3. Methane-methane *W* (*R*) computed in (Ravishanker *et al.,* 1982) and spacefilling diawings of a randomly chosen contact and solvent-separated structure.

the integral equation studies of the hydrophobic effect by Pratt and Chandler (1977) and the simulations on Neon atoms in ST2 water by Pangali *et al.* (1979a,b) indicates that the hydrophobic effect may act over a longer range of distance than previously anticipated *via* these solvent-mediated structures. The structure of the solvent separated forms was studied particularly by Ravishanker *et al.* (1982), who found that the intervening water molecule in the structure is positioned essentially right between the associated methane molecules.

This class of studies has recently been extended to the interaction of phenyl rings in water (Ravishanker and Beveridge, 1985), with the result shown in figure 4. Here as well an oscillatory character is indicated for the calculated potential of mean force. However, the solvent separated minimum does not in this case involve interstitial water molecules, but rather an impingment. Further details on this phenomenon and the relationship to experimental results and the implications thereof promises to be an interesting area for future research studies.

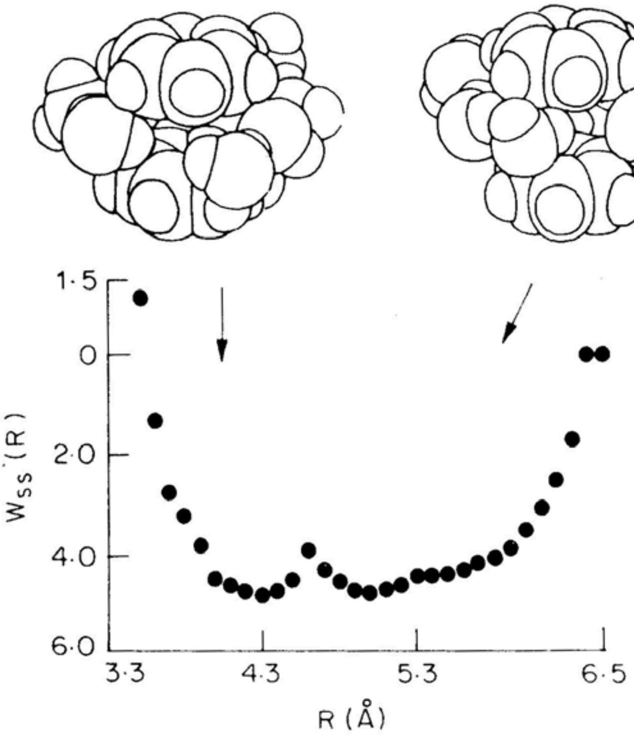


Figure 4. Benzene-benzene W (R)computed (Ravishanker and Beveridge, 1985) and spacefilling drawings of a randomly chosen contact and solvent-separated structure.

Solvent effects on conformational stability

The coupling parameter approach can also be used to calculate conformational free energy of hydration as a function of structural changes in a system, an area of considerable importance in structural biochemistry and biology. The potential of mean torsion for n-butane has been studied by Rebertus, Berne and Chandler (Rebertus *et al.,* 1979), Jorgensen (1982); this sequence of articles has recently been reviewed by Jorgensen (1983). Our studies in this area focus on the conformational preferences of the alanine dipeptide in water and the dimethyl phosphate anion in water. The alanine dipeptide study involved specific structures chosen from among the possibilities suggested in previous theoretical and experimental work: C_5, C_7, α_R and P_{II} (Mehrotra *et al.,* 1984; Mezei *et al.,* 1985); *c.f.* figure 5. Here the various conformations of interest differ in values of the Ramachandran torsion angles ψ and ϕ. We mapped the structural change involving the conformational coordinates ψ and ϕ onto a single ξ by means of the equation

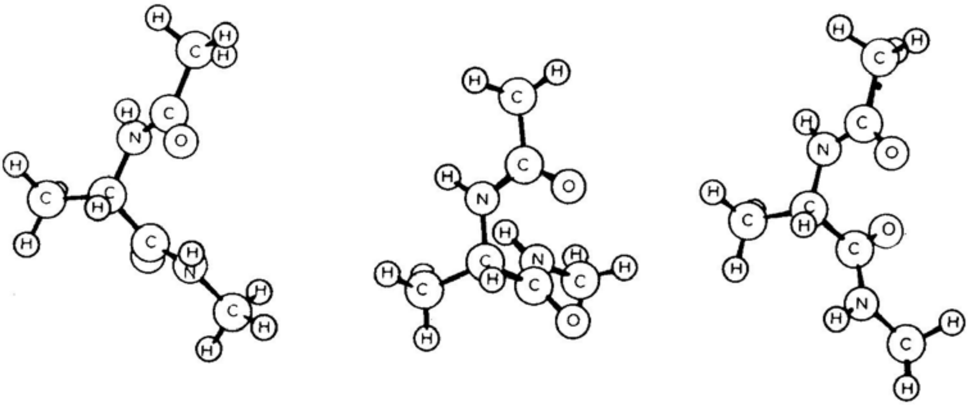


Figure 5. Conformations considered in free energy simulations on the alanine dipeptide: (a)C_7, (b)α_R, (c) P_{II} (from left to right).

$$(\psi, \phi) = (1-\xi)\,(\psi_0, \phi_0) + \xi.(\psi_1, \phi_1), \tag{11}$$

where $\xi = 0$ selects the reference state (ψ, ϕ_0) and $\xi = 1$ selects the state (ψ_1, ϕ_1); here is a correlated conformational transition coordinate. To access computationally tractable sampling procedures in computer simulations it is frequently desirable to map changes in the structure involving many internal coordinates onto a single ξ if possible. In this research, we determined the thermodynamics of hydration for the C_7, α_R and P_{II} conformations of the molecule using conventional mean energy simulations and also free energy simulations using the coupling parameter approach. In this study we mapped the two-dimensional (ψ, ϕ) change into one coordinate. One could proceed in a similar manner to map changes in many dimensions onto one ξ Since the shape of the free energy minimum is not completely determined, this procedure precludes consideration of the full statistical weight problem, but the free energy difference

$$\Delta A = kT\, ln\,[g\,(\xi)_{\xi=0}/g\,(\xi)_{\xi=1}], \tag{12}$$

is available. Use of eq. (12) has the advantage over calculating the mean energy differences from two different simulations in that the statistical uncertainties, resulting from taking a small difference of two large quantities, is eliminated. Note that the ξ used here can be chosen for convenience, since the free energy difference is a state function independent of path. The results indicate that both the α_R and P_{II} conformers are stabilized relative to the C_7 form by hydration, and are both thermally accessible at T = 25°C. Proximity analysis (Mehrotra and Beveridge, 1985) of the results reveals that the origin of the stabilization lies in the differential hydration of the carbonyl group of the amide linkage in the alanine dipeptide (Mehrotra *et al.,* 1980). Experiments based on IR spectroscopic studies reveal the C_7 conformation to be preferred in the nonpolar solvent CCl_4 (Avignon and Lascombe, 1973; Avignon *et al.,* 1973). Nuclear magnetic resonance and circular dichroism spectroscopic evidence for the presence of both α_R and P_{II} conformations of the alanine dipeptide in water at room temperature has been presented (Madison and Kopple, 1980), and thus our simulation results appear to be in general accord with the experiment.

The conformational preferences of dimethyl phosphate can be considered in terms of the phosphodiester torsion angles ω and ω' as *gg, gt* and *tt* (Olson, 1982) as shown in figure 6. The adiabatic potential energy surface for the isolated dimethylphosphate anion is indicated to be relatively flat (Gorenstein *et al.,* 1977). Nevertheless oligonucleotides show a marked preference for the gg and gt forms as shown from crystal hydrate data (Berman and Sheng, 1981). We have carried out mean energy simulations on dimethylphosphate anion in 215 water molecules under periodic boundary conditions (Beveridge *et al.,* 1984). The results indicate that the trans extended form is markedly destabilized by hydration. Free energy simulations using the coupling parameter approach in a manner analogous to that described for the alanine dipeptide are now underway. Preliminary results indicate that the trans extended form is destabilized with respect to free energy as well as mean energy of hydration and thus suggests a possible explanation of why the trans extended form of phosphodiester torsion angles are not found in oligonucleotide crystal hydrates.

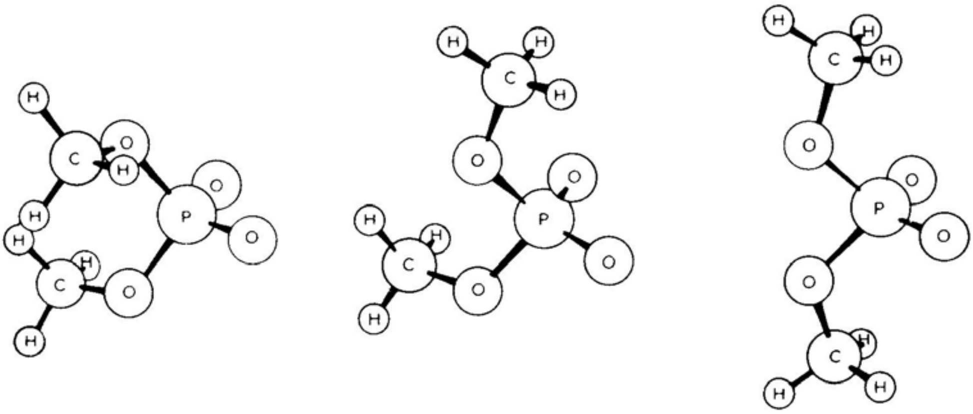


Figure 6. Conformations considered in free energy simulations on the dimethyl phosphate anion (a) *gg*, (b) *gt*, (c) *tt* (from left to right).

Conclusion

We have described here in the coupling parameter approach to free energy simulations provided leading literature references, and reviewed recent studies in the area carried out here at Hunter College. The initial results of free energy simulations using coupling parameter as a topological transition coordinate are generally promising, and we expect that the coupling parameter method will be a useful approach for free energy simulations applied to diverse problems in structural biochemistry. We are currently pursuing free energy simulations applied to the calculation of relative hydration potentials of homologous series of biological molecules, using the coupling parameter as a molecule creation/annihilation coordinate.

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