Use of the Grand Canonical Ensemble in Potential of Mean Force Calculations

H. Resat,*,† M. Mezei,‡ and J. A. McCammon†

Department of Chemistry and Biochemistry, University of California at San Diego, La Jolla, California 92093-0365, and Department of Biophysics and Physiology, Mount Sinai School of Medicine, New York, New York 10029-6574

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Understanding and predicting the thermodynamics of association reactions at the microscopic level requires that it be possible to sample representative configurations of the reactants and solvent as a function of the reaction pathways. Because of geometric effects, certain methodological improvements in molecular simulation techniques are necessary before the reaction thermodynamics of complicated systems such as biopolymers with interlocking shapes can be investigated. Here, we propose the use of the grand canonical ensemble in molecular simulations when the traditional canonical ensemble based methods cannot appropriately account for the confined space effects. The success of the grand canonical ensemble molecular simulations in studying the association reaction profile is shown by testing it on simpler systems. Implications for future work and various possible application areas of the grand canonical ensemble simulations are discussed.

I. Introduction

An important goal of chemical sciences is to understand the relative stability of different arrangements of reactant molecules in solution. For example, the optimal mode of association of an enzyme and an inhibitor molecule (*e.g.*, whether bridging water molecules are involved) can only be determined theoretically by calculating the free energy of the system (potential of mean force) as a function of the solute coordinates. Because of this importance, the potential of mean force has been examined for several classes of systems by various research groups, and these studies have been extensively described in several recent review articles (refs 1−4).

Even though knowing the potential of mean force (pmf) is essential in understanding molecular associations, calculation of pmf's using molecular simulations with explicit solvent models has been rather expensive, especially when the system involves many degrees of freedom. Therefore, the past pmf simulation studies were mostly limited to reactions between small molecules involving only a few degrees of freedom. In this respect, other computationally inexpensive theoretical approaches, such as integral equations,[5] or even continuum models of solvent[6,7] are good alternatives to the simulations. Unfortunately, such techniques are only approximate, and in some cases, their quantitative and/or qualitative predictions can be questionable.[8,9]

In addition to being expensive, computational studies of biochemical systems, such as ligand−receptor systems, are subject to additional difficulties. In many cases, docking of the ligand requires changes in the solvation configuration along a binding channel of the receptor molecule, and the adequate sampling of all the possible configurational changes and the associated time scales increase the required simulation lengths considerably. As was discussed in an earlier report,[10] when the studied system includes confined spaces, such as hydration pockets of crystal hydrates, the conventional simulation methods may not provide adequate sampling. In the earlier crystal hydrate study, this inadequate sampling was overcome by using grand canonical rather than canonical ensemble simulation methods. A similar confined space problem is encountered in many biochemical binding studies as well. For example, as mentioned above, in ligand−receptor reactions, the hydrating water molecules have to be emptied out of the binding channel as the ligand starts to penetrate. The capping of the channel by the ligand may form a confined space, *i.e.*, a water pocket, and this pocket gets smaller as the ligand moves down the channel. As the volume of the water pocket gets reduced, a certain number of water molecules have to move out of the pocket. Using the usual canonical or microcanonical simulation methods, it would be very hard to represent the equilibration of these "squeezed-out waters". Based on its earlier successes[10,11] in overcoming the confined space effects, in this report we propose to use the grand canonical simulations to solve some of the methodological difficulties encountered in the theoretical biochemical binding studies. Although the advantages of using the grand canonical ensemble in pmf calculations would be most apparent in studying the reaction between molecules having interlocking geometries to form confined spaces during the reaction, in this report the application of the proposed idea will be limited to simpler test cases which have been investigated in detail earlier by various groups. The results for the biochemical reaction between a ligand and an enzyme, such as trypsin and benzamidine, for which the method was originally proposed will be the subject of a future report.[12]

An outline of this report is as follows. Section II presents the mathematical details of potential of mean force calculations, the grand canonical ensemble and its implementation into the Monte Carlo simulations, and the details of the computations. The results are presented in section III. The last section, section IV, summarizes our findings and discusses the potential implications for future and ongoing work with special emphasis on biochemical reactions.

II. Theory

A. Potential of Mean Force. The potential of mean force (pmf) is the free energy of a system as a function of selected solute coordinates. The pmf W is related to the distribution probability ρ of states at a fixed reaction coordinate value and is given as[4]

$$\beta W(\mathbf{R}) = -\ln \rho(\mathbf{R}) + C \qquad (1)$$

† University of California at San Diego.
‡ Mount Sinai School of Medicine.
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where $\beta = 1/kT$ and C is a constant. Note that in the above equation the reaction coordinate is represented by **R**, which may be multidimensional. In the simplest case, the reaction coordinate corresponds to the distance between the two labeled particles of the system. If these particles do not have any internal structure, *i.e.*, if they are spherical single interaction site particles, eq 1 reduces to a one-dimensional equation, and for this case, the pmf is related to the pair distribution function, $g(r)$, between the labeled particles[1,5]

$$g(r) = e^{-\beta W(r)} \quad \text{with } W(r) \to 0 \text{ as } r \to \infty \tag{2}$$

It has been well established that calculating the probability distribution function, $\rho(\mathbf{R})$ in eq 1, using a direct Boltzmann sampling in a computer simulation is not practical.[1−4] Therefore, to obtain adequate statistical sampling, the potential of mean force calculations is generally done utilizing non-Boltzmann or biased (umbrella) sampling. Even though the use of a biasing potential alters the Hamiltonian H used in the simulations, it can be shown that[13] the effects of the biasing can be eliminated from the calculated quantities. If the biasing potential is $U_b(\mathbf{R})$, then the pmf is given as[4]

$$\beta W(\mathbf{R}) = -\ln \rho_b(\mathbf{R}) - \beta U_b(\mathbf{R}) + \ln \langle e^{\beta U_b(\mathbf{R})} \rangle_b + C \tag{3}$$

where $\rho_b(\mathbf{R})$ and $\langle \cdots \rangle_b$ respectively are the probability distribution and the ensemble average of the enclosed quantity calculated using the biased Hamiltonian, $H + U_b(\mathbf{R})$.

Common implementations of non-Boltzmann sampling have been either by employing a "guessed" bias potential or by employing a restraining harmonic type potential. As is obvious from eq 3, a uniform sampling of the distribution of states, *i.e.*, ρ_b = constant, would be obtained if an optimal choice for the biasing potential, $U_b(\mathbf{R}) = -W(\mathbf{R})$, could be made. In most cases, the studied pmf does not have a simple form, and it is almost impossible to correctly guess a biasing potential. To avoid such problems and to obtain the optimal sampling, Mezei developed the adaptive umbrella sampling scheme.[14,15] Adaptive umbrella sampling works such that the optimal umbrella sampling potential is self-consistently determined and refined during the molecular simulation, and the simulation is run until an acceptable convergence is obtained. In other words, the biasing potential is updated and adapted at regular intervals during the simulation. The adaptive umbrella sampling idea has already been tested on various systems,[16−18] and it was used in this study.

B. Grand Canonical Ensemble Simulations. Although most of the derivations found in the literature utilize the canonical ensemble, as noted,[19] many aspects of the theory of potential of mean force can be most readily developed within the grand canonical ensemble. Rather than repeating the derivations, we refer the reader to ref 5 for details and present only the final expressions for a single-component system. In the (μ, V, T) grand ensemble, the ensemble average of a quantity O, $\langle O \rangle$, is given as

$$\langle O \rangle = \frac{1}{\Xi} \sum_{N=1}^{\infty} \frac{z^N}{N!} \int d\Gamma \, O e^{-\beta U_N} = \frac{1}{\Xi} \sum_{N} \frac{z^N}{N!} Z_N \langle O \rangle_N \tag{4a}$$

where $Z_N = Z(N,V,T)$ and $\langle O \rangle_N$ respectively are the configuration integral and the ensemble average of O for the corresponding *canonical* ensemble having N particles. In eq 4a, the grand ensemble partition function, Ξ, is given as

$$\Xi(\mu,V,T) = \sum_{N} \frac{z^N}{N!} Z(N,V,T) \tag{4b}$$

where, with Λ denoting the thermal de Broglie wavelength, $z = e^{\beta\mu}/\Lambda^3$ is the fugacity (activity) function. As eq 4 show, in essence, a grand canonical simulation is equivalent to a set of appropriately weighted canonical ensemble simulations. Due to this similarity, the grand canonical ensemble simulation methods were mainly developed by generalizing the existing canonical simulation methods. Further details of grand ensemble simulations may be found in refs 20 and 21.

In this study, we will follow Adams' approach[21] to grand canonical ensemble simulations. Recasting the fugacity in terms of the chemical potential of an ideal gas of particles of the same mass, and the same average number of molecules $\bar{N}$, volume, and temperature, the grand ensemble partition function may be expressed as

$$\Xi = \sum_{N} \frac{1}{N!} e^{NB} \int V^{-N} \, d\Gamma \, e^{-\beta U_N} \tag{5}$$

where the "B" parameter is defined as

$$B = \beta\mu + \ln(V/\Lambda^3) \tag{6a}$$

$$\beta\mu_e = B - \ln \bar{N} \tag{6b}$$

and μ_e is the excess chemical potential [over $\beta^{-1} \ln(n\lambda^3)$, the chemical potential of an ideal gas with number density $n = \bar{N}/V$]. Noting the similarity with the canonical ensemble simulations, Adams developed a grand canonical Monte Carlo (GCMC) simulation scheme in which the move attempts used in canonical ensemble simulations to generate a Markov chain to sample the phase space are replaced with two types of moves: (i) regular moves as in the canonical ensemble, (ii) insertion/deletion moves to allow for fluctuations in the number of molecules. There is no rigorous rule for combining these two move attempts, and in this study, we use a 1:1 ratio; *i.e.*, every regular move is followed by an insertion/deletion attempt.

As eq 6a shows, the B parameter and the chemical potential differ by a constant, and therefore, a constant μ ensemble is equivalent to using a constant B parameter in GCMC simulations. In implementing the GCMC, the B parameter is adjusted at the beginning until the targeted average number of molecules is approximately achieved. After fine tuning, the B parameter is kept constant during the data acquisation, and the average number of molecules is calculated in the same simulation as well. Then the chemical potential can be calculated at the end by using the relation between the excess chemical potential, the B parameter, and the average number of molecules, eq 6b.

C. Reaction Coordinate Range Splitting. It has been well established that calculating the probability distribution function, $\rho(\mathbf{R})$ in eq 1, for the whole range of the reaction coordinate in a single molecular calculation is not practical in most cases.[1,4] Therefore, to obtain adequate statistical sampling, the potential of mean force calculations are generally done as a series of molecular simulations with each sampling a constrained range of the reaction coordinate. This partitioning into smaller subsections is usually achieved by using a constraint potential which functions in exactly the same way as the umbrella sampling discussed above. Although the effects of the constraint potential can be subtracted out at the end, the simulations corresponding to different restrained reaction coordinate ranges have to be matched at the overlap regions. This matching of the split reaction coordinate range pmf's introduces additional

inaccuracies into the calculations. Therefore, it is advantageous to find a biasing potential which would enable the adequate sampling of the entire reaction coordinate range in a single simulation, and such calculations are preferred whenever possible. But, unfortunately, such cases are very rare, and splitting of the reaction coordinate is unavoidable in most instances.

If the reaction coordinate range is partitioned into smaller restrained ranges, then it has to be ensured that the series of molecular simulations correspond to the same thermodynamic state. For the constant temperature, constant volume or constant pressure, and constant N ensembles, the same thermodynamic state can be obtained rather easily for the series of simulations with restricted reaction coordinate ranges. In the grand canonical ensemble, the equality of thermodynamic states requires that the chemical composition composition (*i.e*, the molar ratios) and the chemical potentials of each species in each simulation should be the same.[22] In the GCMC pmf calculations of this report the solute degrees of freedom are fixed to certain values; thus, only the number of solvent molecules is allowed to fluctuate, and there is only one species of solvent (water). In principle, to correctly mimic the macroscopic experimental conditions, the chemical potential of the water used in each simulation should be the same, and this chemical potential should be equal to the one corresponding to a system in which the solute molecule moves are not restricted. In the constant (μ,V,T) GCMC pmf calculations, the number of solute molecules is not allowed to fluctuate, and the employed boundary conditions introduce periodicity by replicating the finite unit cell. Therefore, the equivalency of the chemical composition condition (one solute molecule *vs* $\bar{N}$ waters within the constant volume unit cell) can only be satisfied if the average number of water molecules are kept equal in each simulation. As eqs 6 show, these requirements would be satisfied if the B parameter is kept equal (equivalency of the chemical potentials), and the average number of water molecules is the same (equivalency of the composition) in each window simulation corresponding to different restraints on the solute molecule moves. Note that the latter constraint is due to the replication of the constant volume cell and may be relaxed without significant inconsistency. Also notice that since the artifacts due to the replication will be reduced, this constraint will be better satisfied as the unit cell size is made larger.

It was found, by trail and error, that in GCMC pmf simulations the calculated average number of water molecules for each constrained reaction coordinate range may differ somewhat. These differences in the average number of solvent molecules are small and are mostly due to the unavoidable statistical fluctuations in the number of molecules, but depending on the studied system, the magnitude of such fluctuations may be considerable. Similarly, the characteristic particle number relaxation times may be long,[23] thus requiring lengthy molecular simulations and close monitoring of the convergence characteristics of the runs. Because of this average number density mismatch problem, particular care is needed in partitioning the reaction coordinate into smaller ranges in grand canonical ensemble simulations. Our results, however, showed that for the cases studied in this report the differences in the calculated average number of molecules are rather small (less than 1%), and either the effects of such small thermodynamic state mismatches on the calculated potential of mean forces are negligible or they can be approximately corrected in various ways. One such scheme will be discussed below.

D. Matching of the Thermodynamic States in the Constant (μ, V, T) Ensemble. For simplicity, let us assume that there is only one type of solvent (water), and the reaction coordinate range is split into two sections. Since it is desired that the chemical potential and the average number of waters are to be the same in both runs, the B parameter has to be equal in both simulations. Let us say that the obtained average numbers of water molecules were N_1 and N_2 for the first and the second regions, respectively, and that $N_1 \approx N_2$. Thus, the (excess) chemical potentials for the two runs are slightly different (eqs 6). The chemical potential is a function of the composition (density) at constant volume, so the calculated average number of molecules have to be equalized to obtain the same thermodynamic state between the runs.

As long as N_1 and N_2 are not too different, the following approximation based on the cluster expansion should be a very good one for the potential of mean force calculation between two solute atoms. According to the cluster (or the density) expansion of the potential of mean force (pmf) at infinite dilution, using the hypernetted chain closure approximation the pmf (W) between the solution atoms i and j can be expressed as[5,24]

$$\beta W_{ij} = \beta U_{ij} + \sum_k \rho c_{ik} * h_{kj} \equiv \beta U_{ij} + \beta W_{\mathrm{solv},ij} \qquad (7a)$$

$$\beta W_{\mathrm{solv},ij} = \rho \sum_k c_{ik} * h_{kj} \equiv \rho t_{ij}(r) \qquad (7b)$$

where U is the "direct" pairwise interaction potential between the solute particles, ρ is the solvent number density, and the sum goes over all the sites of the solvent molecules. c_{ik} and h_{ik} are the direct and pair correlation functions between the solute and solvent sites respectively, and * stands for a convolution integral. Equations 7 can be expanded to first order in terms of the solvent density variations to obtain

$$\beta \delta W_{ij}(\rho_0) = \beta \delta W_{\mathrm{solv},ij} = (t_{ij} + \rho_0[\delta t_{ij}/\delta\rho])_{\rho_0} \delta\rho \qquad (8)$$

Equation 8 shows that the solvent density dependence of the pmf may arise either from the explicit linear density dependence or from the implicit density dependence of the solute−solvent direct and pair distribution functions. Assuming that changes in the solute−solvent pair correlation functions with variations in the solvent density, *i.e*., the $[\rho\delta t_{ij}/\delta\rho]_{\rho_0}$ term in eq 8, would not depend on the solvent density, at least for a restricted range of solvent densities, the solvent-mediated contribution to the pmf, $W_{\mathrm{solv},ij}$, can be considered to be a linear functional of the solvent density. Thus, the solvent-mediated contributions to the pmf of the second simulation can be scaled by a factor of N_1/N_2 to achieve the equivalency of the simulation runs. This would assure that both simulations correspond to the same thermodynamic state.

A second and simpler approximation (or lack of correction) would be to perform a series of simulations for restricted reaction coordinate ranges and then to assume that small solvent number density variations will not have much effect on the calculated pmf. Since the potential of mean force is defined up to a constant, the pmf's of the various segments have to be shifted upward or downward to obtain a continuous and best matching pmf. For this reason, the scaling of the solvent contributions by a constant multiplication factor N_1/N_2 would only change the magnitude of the features in the pmf. Therefore, unless there are sharp features, the scaling of the segment pmf's within a couple of percent would not have an important effect on the overall results. As a numerical example, let us consider a chemically typical case and assume that there is a barrier of 3 kcal/mol between contact ion pair and solvent-

separated ion pair configurations and also assume that the barrier is solely due to the solvent-mediated contribution. In this case, a 5% mismatch between the average number densities of the segment simulations would have an effect of approximately 0.15 kcal/mol. For comparison, statistical sampling errors on the order of 1 kcal/mol are not untypical in the potential of mean force calculations. Therefore, for the above example case, the error due to the thermodynamic state mismatch of the partitioned reaction coordinate range is much smaller than the statistical errors and can be neglected.

Notice that the overall shape of the pmf will be available at the end of the simulations. Thus, knowing the features of the pmf would point out whether the corrections due to the thermodynamic state mismatches would be important or not. What this means is that once the simulations are performed, the ratio of the average number of molecules and the specific features in the pmf would enable one to make a good error estimate and, if necessary, corrections may be applied accordingly. As will be shown in the next section, for two LJ particles and for the Na−Cl ion pair in aqueous solution, the particle number mismatch and the resulting error due to the partitioning of the reaction coordinate range are very small, and therefore, the results are left uncorrected.

III. Results

A. Two Lennard-Jones Particles. To test the use of grand canonical ensemble in studying the potential of mean force, the method is initially applied to simpler systems. First, the potential of mean force (pmf) between two identical Lennard-Jones (LJ) particles in aqueous solution is calculated. LJ particles are modeled with a diameter $\sigma = 3.91$ Å and an interaction parameter of $\epsilon = 0.16$ kcal/mol. These parameters are also representative of the united atom model for methyl groups and were taken from the OPLS force field.[25] Water was characterized by the TIP4P model.[26] Solute−solvent interaction parameters were calculated by using the geometric mean mixing rule for both σ and ϵ; $\sigma_{LJ,O} = 3.5115$ Å and $\epsilon_{LJ,O} = 0.1575$ kcal/mol.

The simulation cell had dimensions of $35.15 \times 22 \times 22$ Å^3, and the LJ particles were placed symmetrically around the origin along the *x*-axis (longest axis). Periodic boundary conditions were applied, and the temperature was 298 K. Solute−water interactions were treated with the minimum image boundary condition, and the water−water interactions were truncated with a spherical cutoff at 7.75 Å. Solute and solvent molecule move steps were chosen such that the average acceptance rate was approximately 50%. To accelerate the sampling rate, force biasing[27] as well as a distance-dependent preferential sampling[28] was employed. Since appropriate sampling of the solute−solvent interactions is important in pmf calculations, the calculations employed a scheme in which the selection probability of the solute moves was 8 times higher than that of a solvent molecule. It has been shown by Kincaid and Scheraga[29] that, when the solute−solvent interactions are the major contributor to the investigated quantity, sampling the solute moves more frequently and the preferential sampling of the energetically important waters improves the statistics and the convergence considerably. An adequate acceptance rate, approximately 6.8×10^{-4}, for the insertion/deletion attempts in the grand canonical ensemble simulations was ensured by the use of the cavity bias technique.[30] Note that the obtained rate corresponds to one successful insertion (deletion) attempt in every ∼1500 insertion (deletion) tries. Since the successful insertion/deletion attempts cause large local perturbations in the system, rates much higher than this would not leave enough time for the system to appropriately relax. Therefore, the achieved insertion/deletion rate is large enough, and further increasing this rate would not be recommended.

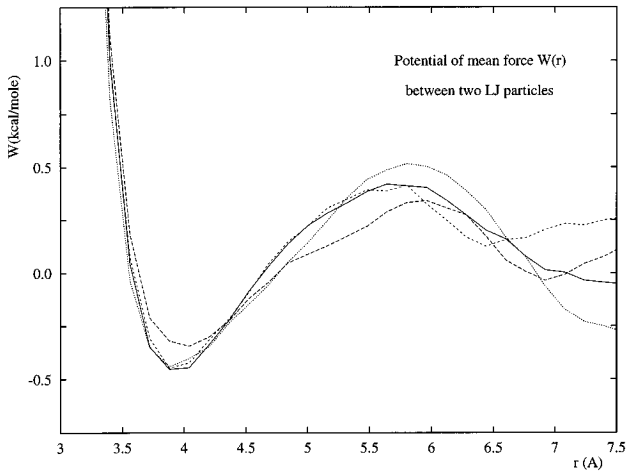

Figure 1. Potential of mean force as a function of the distance between the two identical Lennard-Jones particles. Comparison of different Monte Carlo simulation runs: solid line, canonical ensemble and the reaction coordinate is partitioned into smaller subsections; long-dashed line, canonical ensemble and the whole reaction coordinate is sampled in a simple simulation; short-dashed line, grand canonical ensemble using the whole reaction coordinate; dotted line, grand canonical ensemble using partitioned reaction coordinate range. Reaction coordinate partition ranges and other details are given in the text. Pmf and the distance are in kcal/mol and in angstroms, respectively.

In addition to grand canonical ensemble simulations, canonical (constant NVT) ensemble simulations were also performed for comparison. Overall, four sets of simulations were run: The first one used the canonical ensemble, and the reaction coordinate range was divided into two regions: 3.0−5.4 Å (window 1) and 5.0−8.2 Å (window 2). There were 565 waters and the LJ particle pair inside the unit cell. After equilibration, the simulations for each window were run until good convergence of the pmf was achieved. Due to the cutoof effects, convergence of the simulations at large reaction coordinates is slower and requires longer runs. In this case, convergence of pmf was obtained after running for 20 million (2×10^7) and 28 million steps for the first and the second windows, respectively.[31] The matching of the pmf's for the two windows, *i.e.*, finding the constant in eq 3, was done by matching the window pmf's at the overlapping reaction coordinate points.[14,17] The second simulation also used the canonical ensemble, but this time the reaction coordinate range was increased to 3.0 to 11.0 Å, and the whole range was covered in a single simulation. This was done to test whether the partitioning of the reaction coordinate would be avoidable or not. The runlength of the simulation was 52 million, and the results (Figure 1) proved that it would be possible to use the entire reaction coordinate range in a single simulation, at least for hydrophobic solutes.

To test whether the idea of using the grand canonical ensemble in pmf calculations would be successful or not, the third and the fourth simulations utilized the grand canonical ensemble and repeated the first and the second canonical ensemble calculations. The *B* parameter was chosen to be −3.9, which sets the chemical potential of the waters such that the GCMC simulation unit cell consisted of approximately 565 water molecules on average and the LJ particle pair. Of course, since the grand canonical ensemble was used, the number of waters at a given configuration would fluctuate. In the third simulation, the partitioning of the reaction coordinate was the same as the first canonical ensemble simulation. The GCMC

runlengths were 20 million and 45 million steps (one step = one regular move plus one insertion/deletion attempt) for the first and the second windows, respectively, and the window pmf's were put together by matching the pmf's at the overlapping reaction coordinates. The fourth simulation covered the whole reaction coordinate range and was run for 93 million steps.

Figure 1 shows the good agreement between the canonical and grand canonical ensemble results. Observed deviations among the four simulations are of order 0.25 kcal/mol and such deviations are well within the statistical error bars.[31] Good agreement between the canonical and grand canonical simulations shows that the grand canonical ensemble would be as suitable to study the potential of mean force between particles as the more conventional canonical ensemble. Similarly, the results for the partitioned reaction range simulations and for the simulations using the entire reaction coordinate range agree quite well, too. This finding implies that it might be possible to avoid the partitioning of the reaction range in certain types of pmf studies. As mentioned in section II.C, matching of the adjoining segment pmf's introduces additional possible errors into the pmf calculations. Therefore, the use of the entire reaction range in a single simulation would be advantageous in reducing the errors and should be preferred whenever it is feasible.

Since it is the simplest example of the hydrophobic interactions, the solvation and the association thermodynamics of LJ particles have been studied by several groups.[32,33] Smith and Haymet[33] give an extensive listing of the earlier studies and, by performing very lengthy simulations, detail and discuss the issues important in hydrophobic interaction studies. Although the potential parameters utilized are slightly different, our findings are in good agreement with Smith and Haymet's results. To briefly summarize, after taking into account the 0.18 Å difference in σ_{LJ} of the two studies, calculated positions of the contact pair minimum, roughly 4.0 Å in ours *vs* 3.8 Å in ref 33, match very well. Similarly, the solvent-separated minimum is at approximately twice the $\sigma_{LJ,O}$ distance, 7.0 Å, and the free energy barrier separating these two minima is approximately 1 kcal/mol. Note, however, that the difference in the water models between the two studies and the different treatment of solvent−solvent Coulombic interactions, as well as the different solute−solvent interaction potentials, does not allow for an absolute quantitative comparison. It should also be noted that, due to shorter simulations, the errors in our results are probably larger than those of ref 33.

An additional advantage of the grand ensemble simulation is that the chemical potential of the waters can be obtained in the pmf calculations. Ben-Naim and Marcus[24] define the process of solvation as the process of transferring a molecule for a fixed position in an ideal gas into a fixed position in the liquid at constant temperature and pressure. If this is done in such a way that the number densities in the liquid and gas phases are equal, the obtained quantity would be the Gibbs free energy change of transferring the labeled molecule from the gas phase into the liquid.[34] Therefore, if the intramolecular partition function of the transferred molecule is the same in both phases, what is defined as the Gibbs free energy of solvation by Ben-Naim and Marcus is equivalent to the excess chemical potential μ_e in eqs 6. However, since a constant volume rather than a constant pressure ensemble is used in this study, a correction term is required to convert from the Helmholtz free energy into the Gibbs free energy to account for the difference between the simulation pressure, *p*, and the standard state pressure of 1 atm. For this, the thermodynamic formula

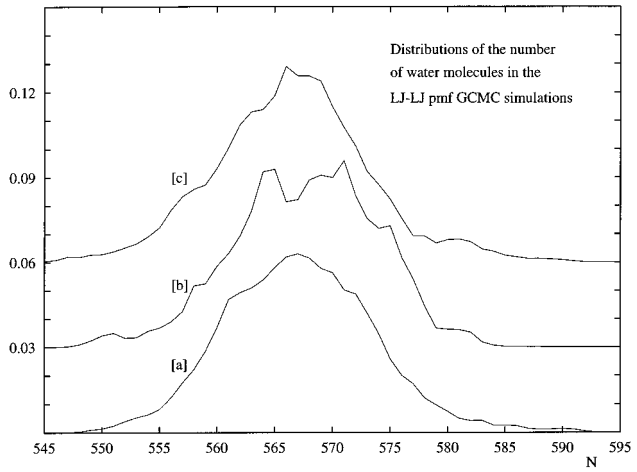

Figure 2. Distributions of the number of water molecules in the grand canonical MC pmf simulations between two identical Lennard-Jones particles, the third and the fourth runs of the text. [a] GCMC simulation utilizing the entire reaction range in a single simulation, the fourth run. Also shown are the corresponding results of GCMC simulation with split reaction coordinate range, the third run: [b] first window (3.0−5.4 Å) and [c] second window (5.0−8.2 Å). [b] and [c] are shifted by 0.03 and 0.06, respectively.

$$\left(\frac{\partial p}{\partial \mu}\right)_{t,V} = \rho \tag{9}$$

may be utilized. Assuming a small compressibility, *i.e.*, an almost constant density as a function of the pressure, establishes the correction term as $V\Delta p/N$. Substituting the value of the *B* parameter and the obtained average number of waters into eq 6b, and using eq 9 to correct for the ensemble differences, we calculated the chemical potential of the TIP4P model as −6.14 kcal/mol. This value is in good agreement with the Jorgensen *et al.*[35] result of −6.1 kcal/mol and with experiments,[34] −6.324 kcal/mol. Even though what is calculated in this study is the chemical potential of the TIP4P model water in the presence of a pair of perturbing LJ particles, the large size of the unit cell and the weak strength of the perturbing LJ particles should not have a sizable impact on the chemical potential of the waters. In fact, for a truly infinitely dilute solution with no finite size effects, the calculated water chemical potential should approach that of bulk water. Since the unit cell used in this study is fairly big, the simulated system mimics the inifinite dilution solution with reasonable accuracy. For a consistency check, the chemical potential of bulk TIP4P water was also calculated in a separate simulation. Calculated chemical potentials of bulk water and of water in LJ−LJ solution were different by only 1%, supporting the above-stated expectation.

Figure 2 reports and compares the distributions of the number of water molecules in the third and the fourth simulations. For clarity, the results of the third simulation (windows 1 and 2) are shifted upward. As can be seen, the distributions of number of waters during the simulations using the entire reaction coordinate range (fourth simulation) or using a partitioned reaction coordinate range (the first and the second windows of the third simulation) are very similar. Although it is somewhat noisy, the overall shape of the distribution function closely resembles that of a Gaussian distribution with a dispersion of 6−7 molecules.

As mentioned in section II.D, an additional concern in the grand canonical ensemble simulations with parttioned reaction coordinate range is the matching of the thermodynamic states

of the various simulations. For the LJ−LJ pmf case, the thermodynamic states are quite equivalent. The calculated average number of waters in the first and the second windows of the third simulation were 567.6 and 566.5, respectively. These are off from the targeted average number of waters 565 by less than three molecules.[36] Since the differences are quite small, less than 0.5%, and since the calculated pmf does not have any sharp features, as discussed in section II.D, the grand canonical ensemble results can be approximately corrected for an appropriate comparison with the canonical ensemble calculations. Since the direct interaction potential between the LJ particles is short-ranged, the features in pmf at large distances are largely due to the solvent-mediated contributions. Reading from Figure 1, we see that the magnitude of the features in the pmf such as the barrier between the contact and solvent-separated minima are of the order 1 kcal/mol. Thus, a 0.5% correction would contribute about 0.005 kcal/mol, and an error of such magnitude can be safely ignored in the pmf calculations. The fourth simulation, the grand canonical ensemble simulation sampling the entire reaction coordinate range of 3−11 Å, actually consisted of two separate simulations starting with different initial configurations and with runlengths of 45 million and 48 million steps. The average number of waters obtained for each run respectively were 566.5 and 567.9, giving an overall average of 567.2 waters. This is off from the aimed number of waters only by 2.2 molecules, corresponding to a 0.4% correction of solvent-mediated contribution to the pmf. Similarly, the average number of water molecules mismatch between the two separate runs of the fourth simulation is only 1.4 molecules, which is equivalent to a correction factor of 0.25%. Such corrections should be very insignificant, and therefore no correction was applied to the reported results.

B. Sodium Chloride. The apparent success of the grand canonical ensemble simulation for studying the pmf of two LJ particles prompted us to further test the method by studying another simple system. The association of Na^+ and Cl^- in water was chosen as our second test case. Notice that, unlike LJ particles, this case involves fully charged ions and cutoff effects are expected to be much more important. Actually, in their recent work, Friedman and Mezei[18] found that the way the interaction potentials are handled can have significant effects on the calculated pmf. They investigated the cutoff effects in detail by performing a wide range of simulations. At large distances, the calculated Na−Cl pmf looks very different than what is expected on physical grounds.[18] To further their investigation, the interaction potential parameters were kept the same here, namely Jorgensen's TIPS2 water model[37] for water−water interactions and his potentials for the ions;[38] $\sigma_{Na} = 1.897$ Å, $\epsilon_{Na} = 1.607$ kcal/mol, $\sigma_{Cl} = 4.417$ Å, and $\epsilon_{Cl} = 0.118$ kcal/mol were used. The geometric mean mixing rule for both σ and ϵ was employed. The simulation conditions were the same as in the LJ−LJ pmf case except that solute−water interactions were treated with the minimum image boundary condition, and the minimum images were determined with respect to the center of mass of the Na^+−Cl^- ion pair. The water−water interactions were truncated with a spherical cutoff at 11 Å. Further details of the simulation setup may be found in ref 18.

The reaction coordinate (distance between the ions) was divided into five regions: from 2.0 to 2.6 Å (window 1), from 2.5 to 3.5 Å (window 2), from 3.2 to 5.5 Å (window 3), from 4.6 to 6.3 Å (window 4), and from 5.9 to 8.0 Å (window 5). With the choice $B = -2.7$ for the chemical potential, the unit cell consisted of 565 water molecules on average and the ion pair. After equilibration, adaptive umbrella sampling GCMC simulations for each window were run until sufficient convergence is achieved. Runlengths of the segment simulations and the calculated average number of waters were 566.1 (25 million steps), 564.1 (36 million steps), 566.3 (48 million steps), 565.3 (50 million steps), and 566.1 (58 million steps) for windows 1 to 5, respectively. Note that these average number of waters are very close to each other (with a maximum deviation of 0.4%), and therefore, as in the LJ−LJ pmf case, the thermodynamic state mismatch corrections were unnecessary and were simply omitted. Using the B parameter value, the average number of molecules, and the small pressure correction using eq 8, the chemical potential of the TIPS2 model water (when the ion pair is present) was −5.44 kcal/mol. This is 0.7 kcal/mol larger than that of the chemical potential of TIP4P model water.

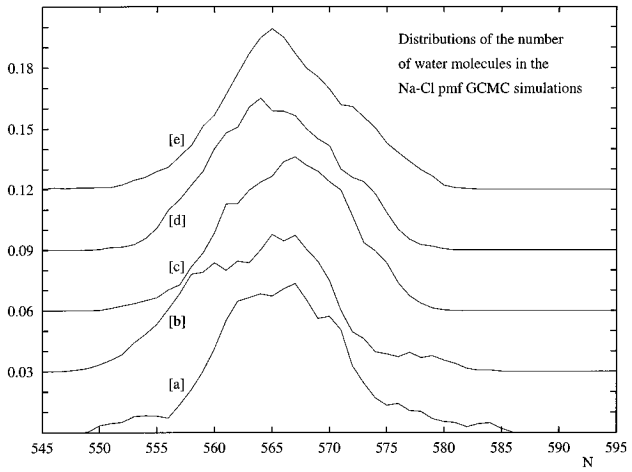

Figure 3. Distributions of the number of water molecules in the grand canonical MC pmf simulations between sodium and chloride ions. Comparison of different window simulations: [a] first window (2.0−2.6 Å), [b] second window (2.5−3.5 Å), [c] third window (3.2−5.5 Å), [d] fourth window (4.6−6.3 Å), and [e] fifth window (5.9−8.0 Å). [b], [c], [d], and [e] are shifted by 0.03, 0.06, 0.09, and 0.12, respectively.

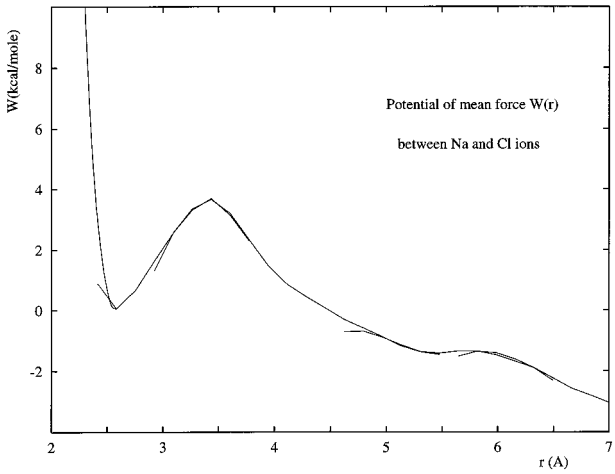

Figure 4. Potential of mean force as a function of the distance between sodium and chloride ions. Pmf and the distance are in kcal/mol and in angstroms, respectively.

The distributions of the number of water molecules in each window simulation are reported in Figure 3. The distribution functions of different window simulations agree with each other reasonably well. Another observation is that the distributions of the number of water molecules in the Na−Cl pmf (Figure 3) and in the LJ−LJ pmf calculations (Figure 2) are very similar.[39] The partitioning of the reaction coordinate range or changing the solutes would only affect the solvent molecules in the vicinity of the solute; thus, only a small percentage of solvent

molecules would be affected. Therefore, the artifacts due to reaction coordinate partitioning or due to the periodicity stemming from the boundary conditions would be less and less important as the system unit cell size gets larger. Since the unit cell used in this study is fairly big, the simulated system mimics the infinite dilution solution with enough accuracy. Therefore, the observed agreement between the different window runs of Na−Cl and of LJ−LJ pmf calculations is not that surprising and actually is to be expected.

Comparing the presented grand canonical ensemble simulation results, Figure 4, with earlier canonical ensemble studies, we find that the agreement for the contact pair minimum distance, 2.7 Å, and the agreement for the barrier height, about 3.7 kcal/mol, is fairly good.[40] However, as in ref 18, our results at large ion separation distances seem to disagree with the other studies. Although its position, 5.4 Å, agrees well with other studies,[40−42] the solvent-separated minimum is not pronounced in our results and appears only as an inflection point. Also, the decrease in the pmf at larger distances is most probably physically incorrect. Since even small changes in the interaction potential parameters,[41] the employed water model,[42] different treatment of the boundary conditions,[43] and the different cutoff schemes and values[18] can have significant effects on the calculated pmf, quantitative disagreement between different pmf studies is not unusual. A very good example of this is the differing results in different studies for the relative stability of the contact pair and the solvent-separated pair Na−Cl ion complexes in aqueous solution.[40] The relative stability found in different studies varies considerably, and the main cause of the differences among the studies is the variation of the calculated pmf's around the solvent-separated minimum distances. This is due to the fact that the calculated pmf around the solvent-separated minimum and at larger distances seems to be particularly sensitive to the above listed effects.[18,43] Not surprisingly, this is the region that we find disagreement with the other studies. Since the agreement around the contact minimum is fairly good, and since our aim in this work is not to calculate the most accurate pmf between Na and Cl or between two LJ particles, but to illustrate that the grand canonical simulation ensemble idea is reasonable, we do not attribute too much significance to the disagreement between our results and the other studies concerning the solvent-separated minimum. Extending our simulation run lengths did not change the results, which further shows that observed disagreements are most likely due to the cutoff effects rather than improper sampling in the simulations. In this regard, approaches to make the simulated system effectively infinite in size such as Ewald summation,[44] particle−particle particle−mesh technique,[45] and fast multipole algorithm,[46] or approaches to incorporate the surrounding solvent as a dielectric continuum using the Poisson−Boltzmann equation[47] or the generalized reaction field formalism[48] should prove useful. Further discussion of the advantages and disadvantages of different boundary conditions may be found in ref 18.

IV. Summary and Discussion

Grand canonical Monte Carlo simulations are used to calculate the potential of mean force between two Lennard-Jones particles and to calculate the potential of mean force between a sodium cation and chloride anion, all in water. Different simulations were performed to show the equivalency and compare the efficiency of different simulation approaches. Comparison of the pmf results between two LJ particles when the reaction range is split into smaller subsections, and when the whole range is used in a single simulation, shows that it might be possible to avoid the partitioning of the reaction coordinate range in certain types of simulations. When possible, the use of the whole reaction range is advantageous in the sense that the errors due to the matching of the potential of mean force segments are eliminated. One implication of this finding is that results obtained from the simulation using the entire reaction coordinate range can be used as a benchmark to compare different ways of matching the results of the partitioned reaction coordinate simulations. For example, it is still an open question[14,17] whether matching the segment potential of mean forces or matching the segment sampling probabilities is the better way of putting the results together. The availability of the results without matching errors for the whole range would establish good benchmark references for comparing different approaches. However, it should be kept in mind that proper sampling of the phase space in simulations covering the entire reaction coordinate range may need extremely lengthy runs, and such simulation should be utilized with utmost caution.

As discussed in sections II.C and II.D, when the reaction coordinate is partitioned into smaller parts, it has to be ensured that the simulations for each subregion correspond to the same thermodynamic state. The results for the studied cases showed that thermodynamic state mismatches are unimportant at least for the systems examined in this report. Even though it was not utilized in this report, an approximation scheme to achieve thermodynamic state equivalency between different simulation runs was introduced. This correction scheme, section II.D, is based on the cluster expansion of the potential of mean force and should be a very good approximation when the observed density differences between the simulations are small. As commented, the correction can be applied at the end and, since the features of the calculated potential of mean force will be known by then, an accurate error assessment due to the approximate correction can be done even before the corrections are applied.

The availability of such correction schemes and the observed good agreement between canonical ensemble and grand canonical ensemble simulations are very encouraging and show that grand canonical ensemble simulations are well suited for pmf calculations. The main advantages of grand canonical ensemble simulations will be much more apparent when certain geometrical shape effects make the corresponding canonical ensemble simulation methods unsuitable. As stated in the Introduction, it would be difficult to study certain biomolecular association reactions using conventional canonical ensemble methods, and therefore, grand canonical ensemble methods should prove to be particularly useful in biochemical association studies. In ongoing work, we are investigating the association of an example enzyme−ligand system; these results will be communicated in a future report.

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