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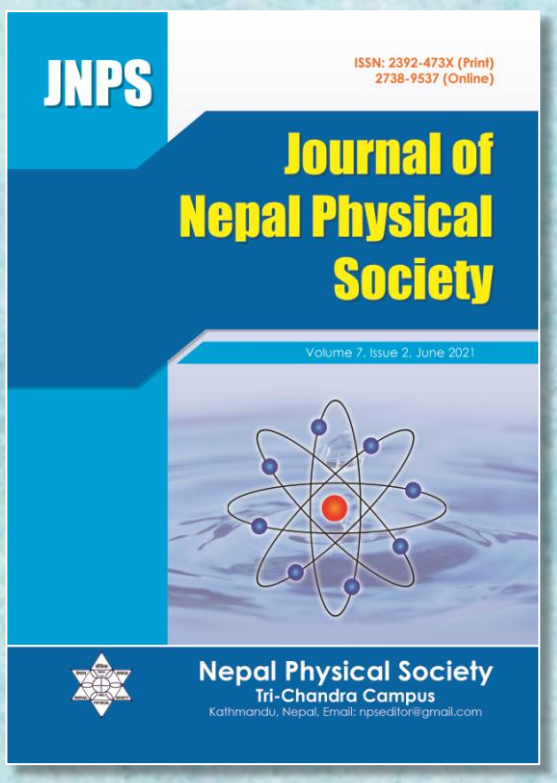

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Solvation Free Energy of Protonated Lysine: Molecular Dynamics Study

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ABSTRACT

In the present work, we have used an alchemical approach for calculating solvation free energy of protonated lysine in water from molecular dynamics simulations. These approaches use a non-physical pathway between two end states in order to compute free energy difference from the set of simulations. The solute is modeled using bonded and non-bonded interactions described by OPLS-AA potential, while four different water models: TIP3P, SPC, SPC/E and TIP4P are used. The free energy of solvation of protonated lysine in water has been estimated using thermodynamic integration, free energy perturbation, and Bennett acceptance ratio methods at 310 K temperature. The contributions to the free energy due to van der Waals and electrostatics parameters are also separately computed. The estimated values of free energy of solvation using different methods are in well agreement with previously reported experimental value within 14 %.

Key words: Molecular dynamics simulations, Solvation free energy, Free energy perturbation and Thermodynamic integration.

1. INTRODUCTION

Solvation free energy plays a central role in protein folding, protein function and molecular recognition [1, 2]. Theoretical and computer simulation inspection on the thermodynamic properties of amino acids and the role of free energy in particular, in this context, become very important in a broad range of fields from chemistry, biology, and pharmaceuticals. Such studies can pave the way for identification of pharmacological targets as well as in the drug discovery [3]. Since most of the biological processes happen in an aqueous solution, this is why solvation free energy is equivalently referred to as hydration free energy that originates from the interactions between the solute and solvent i.e., water. Additionally, free energy calculations often provide an efficient route to estimate kinetic and dynamic characteristics of bio-chemical and physical processes, such as solubility, reaction rates, partition coefficients, associations, dissociations, and binding constants [4].

Number of experimental studies has been carried out to determine the solvation properties of amino acids in past decades [5–8]. Because of the very different physico-chemical properties among the naturally occurring amino acids, the experimental techniques may not be free from intrinsic errors. Therefore, it is highly desirable to complement experimental studies with theoretical approaches using molecular dynamics simulations with explicit solvent molecules [9]. Several researchers performed the molecular dynamics simulations [10–12] and even monte carlo simulations [13] to determine the solvation free energies of amino acids in different solvent environments using variety of available force fields. These calculations show that it is possible to reproduce the experimental solvation free energies by modeling different interactions between the solute and solvents. Motivated from aforementioned studies, we have chosen the protonated lysine system for the calculation of solvation free energy in aqueous solution.

Lysine, an essential amino acid used in biosynthesis of proteins, is harvested from external food stuffs. Also, it is required for growth, tissue repair and improves immune system [14]. Going

through the literature for our case, the experimental value of the solvation free energy of protonated form of lysine in water is known to be 277.80 kJmol $^{-1}$ [15] and value 246.22 kJmol^{-1} have been predicted with use of molecular dynamics simulations [16]. To our best knowledge, there are no simulations have been done in order to estimate the solvation properties of lysine in it's protonated form using Molecular Dynamics (MD) simulations with implementation of variety of Free Energy Perturbation (FEP) and Thermodynamic Integration (TI) based methods in aqueous solution. In this work, we have carried out MD simulations to estimate free energy of solvation of the protonated lysine in aqueous medium. The simulations have performed taking four different water models: TIP3P, SPC, SPC/E and TIP4P. Then, the solvation free energy has been estimated using TI and FEP methods. The obtained results are compared with available previously reported data. A standard comparison is made between each method with potential sources of errors. The solvation free energies due to change in van der Waals parameters and electrostatic parameters are also computed individually.

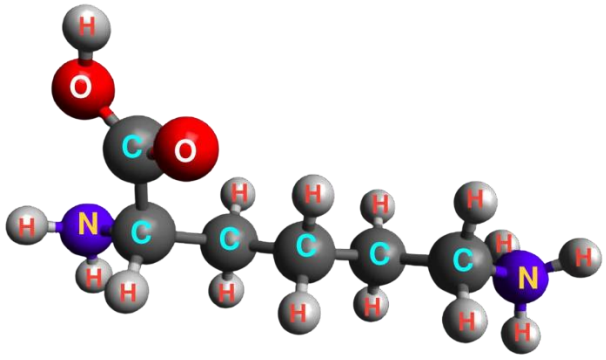

Fig. 1: Protonated lysine molecule.

2. THEORY

The free energy difference between two states say A and B of a system can be calculated using thermodynamic integration (TI) method as [17]:

$$\Delta F_{AB} = \int_{\lambda=0}^{\lambda=1} \left\langle \frac{\partial U}{\partial \lambda} \right\rangle_{\lambda} d\lambda \ldots (1)$$

Here, the parameter λ is used to define intermediate states between initial and final states. For this the potential energy is defined such that it is also a function of the coupling parameter λ. The equation (1) can be evaluated for the ensemble average at a number of discrete λ -points by performing series of simulations for each chosen λ-point. We then use numerical integration to determine the integral.

When the free energy difference between two states of a system is small, another approach based on perturbation, called free energy perturbation (FEP) method, can be used [18]. According to this method, the free energy difference, is defined by [19].

$$\Delta F_{AB} = k_B T ln \left\langle e^{-\beta[U(\lambda_B)-U(\lambda_A)]} \right\rangle_{\lambda_A} \ldots (2)$$

Here, $\beta = (k_B T)^{-1}$, where k_B is the Boltzmann constant and T is absolute temperature. In this method, to obtain convergence, significant overlap of the low energy regions of the two ensembles is required.

The asymmetric biased arises in equation (2) due to the configuration being sampled either via λ_A or λ_B can be removed by so called Bennett Acceptance Ratio (BAR) method [20]. The BAR method requires sampling and energy evaluation of the system configurations from both states to estimate the free energy difference [21].

$$\sum_{i=1}^{n_i} \frac{1}{1+exp\left[ln\left(\frac{n_i}{n_j}\right)+\beta(\Delta U_{ij}-\Delta A)\right]} = \sum_{j=1}^{n_j} \frac{1}{1+exp\left[ln\left(\frac{n_j}{n_i}\right)+\beta(\Delta U_{ij}-\Delta A)\right]} \ldots (3)$$

The expression (3) minimizes the free energy variance and makes BAR more efficient [22]. In order to increase the overlap between each pair of end states, free energy differences are usually calculated by introducing number of intermediate states in addition to the two end states. A multistate extension of BAR, called the Multistate Bennetts Acceptance Ratio (MBAR), has been proposed [23]. In this approach, a series of weighting functions are derived to minimize the uncertainties in free energy differences between all states considered simultaneously. For the case when only two states are considered MBAR reduces to BAR. Among all the methods discussed here, MBAR has the lowest variance, and is apparently the most decisive estimator of the solvation free energy calculations [23, 24].

3. COMPUTATIONAL DETAILS AND METHODOLOGY

In this work, lysine in protonated form was used as solute and four different models of water: transferable intermolecular potential with 3 points (TIP3P), simple point charge (SPC), extended simple point charge (SPC/E) and transferable intermolecular potential with 4 points (TIP4P) were used as solvent [25–27]. The geometry of solute was mimicked by using Optimized Potentials for Liquid Simulations-All Atom (OPLS-AA) force field parameters [28]. To prepare the simulation cell, a cubical box of size 3 nm was taken. The solute was placed at the center of this box and filled with 875 water molecules at the experimental density. Simulation was performed under periodic boundary conditions (PBC) at 310 K using GROMACS (5.1.2) package [29, 30].

The approach to find the solvation free energy is by turning off the interactions; bonded and non-bonded, between the solute and the solvent molecules. For our case, we only manipulate non-bonded interactions. In determining the solvation free energy, we can use the fact that free energy is a state function; it is independent of the path taken for the transformation going from state A to state B. For this, we define the potential of the system as a function of two parameters λ_{vdW} for van der Waals potential, and λ_{ele} for electrostatic potential. Thus, the complete description of our system for state A ($\lambda = 0$), where there was interaction (couple) between the solute and solvent, and for state B ($\lambda = 1$), where there was no interaction (decouple) between the solute and solvent. This was done in our case by scaling the system by taking 21 windows for values of λ between 0 and 1. The 21 different values of coupling parameter for Coulombic and Lennard-Jones interactions were defined as: λ_C = 0.00, 0.10, 0.20, 0.30, 0.40, 0.50, 0.60, 0.70, 0.80, 0.90, 1.00, 1.00, 1.00, 1.00, 1.00, 1.00, 1.00, 1.00, 1.00, 1.00, 1.00 and λ_{LJ} = 0.00, 0.00, 0.00, 0.00, 0.00, 0.00, 0.00, 0.00, 0.00, 0.00, 0.00, 0.10, 0.20, 0.30, 0.40, 0.50, 0.60, 0.75, 0.80, 0.90, 1.00 respectively.

At first, the energy minimization was carried out using steepest-descent method [31] with tolerance force set to 10 kJ mol^{-1} nm^{-1}. To generate the initial velocities Maxwell-Boltzmann distribution was used. The Brownian dynamics friction coefficient was set to zero with random speed -1. The leap-frog stochastic dynamics integrator [32] was used to integrate the equations of motion. The temperature was kept constant at 310 K by using the Langevin thermostat [33]. The pressure was maintained constant by coupling to a reference pressure of 1 bar using the Parrinello-Rahman barostat [34] with compressibility 4.5×10^{-5} bar^{-1}. For the simulations, the coupling time for both thermostat and barostat was set to1 ps. The time step used in the simulations was 2 fs throughout. A 5 ns simulation was performed to equilibrate each of the systems before the start of the actual free energy calculations.

In this study, a neighbor list of 1.2 nm updated every twenty steps was used for the short range interactions. Particle Mesh Ewald (PME) was used to evaluate the Coulombic interactions, with a real space cutoff of 1.2 nm and a PME order of 6 [35]. The Fourier spacing was chosen to be as close to 0.12 nm as possible given the box size and the need for integer numbers of grid points. The distance for van der Waals cutoff was set to 1 nm. All the bond angles were constrained using the LINCS algorithm [36]. For the calculation of free energies, we gave the final production run of 5 ns with time step 2 fs. During the production run, the system was coupled only using temperature. In the TI, in order to remove the singularities in the potentials, we used λ dependence of potentials; the soft-core potential [37].

4. RESULTS AND DISCUSSION

The solvation free energy of protonated lysine in water has been estimated at 310 K using TI and FEP methods [38]. In TI method, the free energy change for a path composed of 'm' states is computed as a weighted sum of the ensemble averages of the derivative of potential energy function with respect to coupling parameter λ. There are different approaches available for numerical integration of TI. But in our calculation, we have implemented TI-1 and TI-3 which use the trapezoidal rule (a first-order polynomial) and a cubic spline respectively. The nature of these TI methods depends on the nature of the curve being integrated and hence, it depends on underlying data and the shape of the $\partial U/\partial\lambda$ the alchemical path chosen. Perturbation based methods include a broad range of techniques. The direction dependent transformation of FEP originates from under-sampling the tail regions of the potential distributions, which results biased free energy [39]. We have used BAR method that uses samples of potential energy in both direction to obtain the minimum free energy variance. In BAR, the free energy change between two adjacent states is computed to yield the minimum variance and gives

data for single pair of states, while another class of BAR is MBAR that finds the best estimation of free energy changes between all states simultaneously by optimizing the matrix of the ΔA variance.

The BAR method provides a maximum likelihood estimation of the free energy that is given by the samples from the two states. The BAR requires significantly less phase space overlap between these states in order to converge results as compared to other methods [40, 41]. Note, however, that BAR requires sampling and energy evaluation of the system configurations from both states to estimate the free energy difference. As phase space overlap affects the reliability of the estimate, free energy differences are most often calculated by simulating several intermediate states in addition to the two end states, in order to increase the overlap between each pair of states. A multistage extension of BAR method called as MBAR has been devised [23]. In this approach, a series of weighting functions are derived to minimize the uncertainties in free energy differences between all states considered simultaneously. MBAR reduces to BAR when only two states are considered.

To estimate the solvation free energy of our system, we have first plotted the ∂U/∂λ as a function of coupling parameter λ.

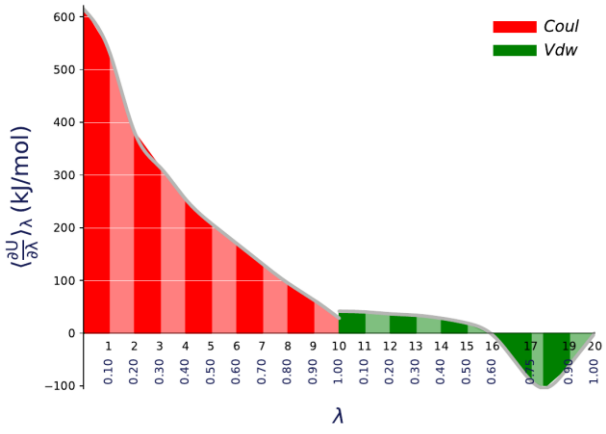

Fig. 2: Variation of ∂U/∂λ as a function of λ taking TIP3P water model as solvent.

Figures 2, 3, 4 and 5 represent the variation of $\left\langle \frac{\partial U}{\partial \lambda} \right\rangle$ as function of coupling parameter λ taking TIP3P, SPC/E, SPC and TIP4P water models as solvent respectively. The estimated values of solvation free energy of protonated lysine in four different water models: TIP3P, SPC, SPC/E and TIP4P calculated from TI, TI-CUBIC, BAR and MBAR with previously reported experimental value are presented in the Table (1).

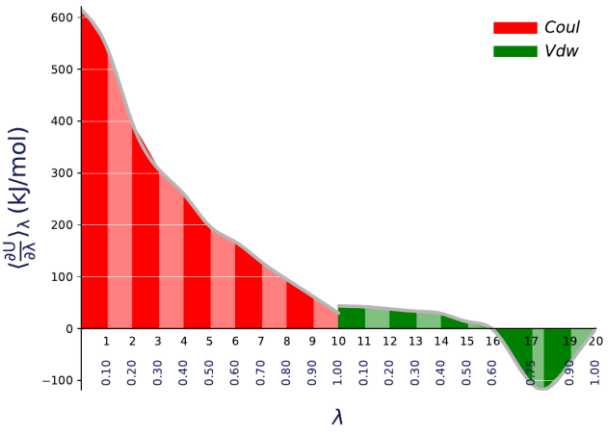

Fig. 3: Variation of ∂U/∂λ as a function of λ taking SPC/E water model as solvent.

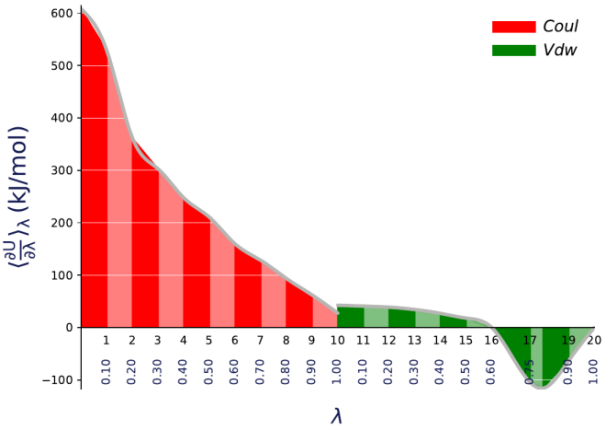

Fig. 4: Variation of ∂U/∂λ as a function of λ taking SPC water model as solvent.

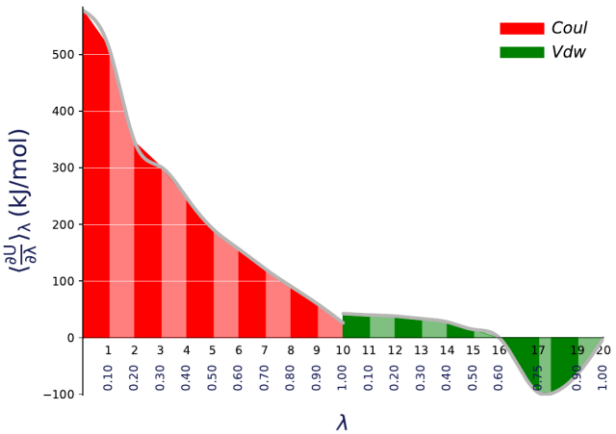

Fig. 5: Variation of ∂U/∂λ as a function of λ taking TIP4P water model as solvent.

Table 1: Estimated values of solvation free energies for protonated lysine in different water models: TIP3P, SPC/E, SPC and TIP4P at 310 K temperature using TI, TI-CUBIC, BAR and MBAR methods with previously reported experimental value.

Water models	Interactions	TI (kj-mol^{-1})	TI-CUBIC (kj-mol^{-1})	BAR (kj-mol^{-1})	MBAR (kj-mol^{-1})	Expt.[15] (kj-mol^{-1})
TIP3P	Coulomb	247.36±0.35	247.23±0.35	247.37±0.22	247.71±0.24	277.80
	vdW	-5.45±0.18	-5.61±0.20	-4.53±0.16	-4.42±0.18	
	Total	241.92±0.40	241.62±0.40	242.84±0.27	243.29±0.38	
SPC/E	Coulomb	247.22±0.	247.07±0.40	247.52±0.26	247.51±0.26	
	vdW	-8.44±0.24	-8.58±0.27	-6.91±0.22	-6.96±0.2.40	
	Total	238.78±0.47	238.49±0.48	240.60±0.34	240.55±0.36	
SPC	Coulomb	241.12±0.61	241.02±0.63	238.88±0.35	238.82±0.41	
	vdW	-7.42±0.19	-7.56±0.22	-6.14±0.19	-6.09±0.21	
	Total	233.70±0.64	233.46±0.67	232.73±0.40	232.74±0.46	
TIP4P	Coulomb	232.70±0.43	232.76±0.42	238.33±0.27	240.90±0.17	
	vdW	-6.08±0.22	-6.61±0.25	-5.08±0.20	-4.99±0.22	
	Total	226.62±0.49	226.14±0.49	233.25±0.33	235.91±0.27	

Since the process we conducted in this work was the decoupling of protonated lysine in water, so the positive sign appears in each values. The reverse process is also possible. For our simplicity we just take the absolute value. The free energy change from $\lambda = 0$ to $\lambda = 1$ is simply the sum of the free energy changes of each pair of neighboring λ simulations. For all four water models: TIP3P, SPC, SPC/E & TIP4P, we have also estimated the individual contributions of electrostatic and vdW interactions to the solvation free energy. From the Table 1, it is seen that the contribution of vdW to solvation free energy is negative but of Coulombic interaction is positive. Also, in the current alchemical transformation protocol, the vdW component of the solvation free energy has found to be far less than that of the electrostatic component. From this observation, we have concluded that the solvation free energy of protonated lysine in water is solely due to the electrostatic component. Also, it is seen that the estimated value of solvation free energy is closer to the experimental value for TIP3P water model than that of other models.

For the TIP3P model, the estimated value of solvation free energy using MBAR method is in close agreement to the previously reported value rather than the values estimated using other methods. The estimated value using MBAR is in 12% agreements with the previously reported value. Also, other methods show a maximum difference of > 2 kJ/mol from the value estimated using MBAR method. We also observed from the Table 1 that the solvation free energy in each water model has almost same values using different methods indicated that λ spacing was sufficient for our sampling distributions which were found to be overlapped properly.

Furthermore, the high value of solvation free energy suggests that the lysine in its protonated form is highly soluble in water. This hydrophilic nature of lysine can be explained as follows: Lysine is a simple basic amino acid. In spite of a long and potentially hydrophobic chain, it has a basic NH_2 at the end of side chain. In its basic deprotonated form, lysine is neutral and hydrophilic; however, if found in physiological pH, lysine will pick up an H^+ from solution to form an NH_3^+ salt. Salts are charged and therefore definitely hydrophilic.

5. CONCLUSIONS AND CONCLUDING REMARKS

In this work, we performed molecular dynamics (MD) simulation to estimate the solvation free energy of protonated lysine in aqueous medium. OPLS-AA force field parameters and four different water models: TIP3P, SPC, SPC/E and TIP4P were used during the simulations. We have used TI, TI-cubic, BAR and MBAR methods to estimate the salvation free energy. It has been observed that the estimated values of free energy of solvation of protonated lysine with TIP3P water as solvent using different methods have closer values with experimental value in comparison to other models. The estimated values of free energy of solvation of protonated lysine in TIP3P water model at 310 K temperature are 241.92, 241.62, 242.84 and 243.29 in kJ/mol from TI, TI-cubic, BAR and MBAR methods respectively. Obtained numerical values of free energies demonstrated that all these methods are able to reproduce experimental free energies of solvation in water solvent. We have also analyzed the contribution of van der Waals (vdW) and electrostatic interactions to estimate the free energy of solvation; and it has been observed that the electrostatic interaction has major contributions to the solvation free energy. The estimated values of free energy of salvation in TIP3P water model using different methods are in agreement with previously reported experimental value within 14%. To extend this work in near future, we plan to study the solvation free energy of lysine in other solvent environment. Further, we plan to study the solvation free energy of lysine peptides and observe the effect on solubility with increase in chain length of solute.

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