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Umbrella sampling of proton transfer in a creatine–water system

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ABSTRACT

Proton transfer reactions are among the most common processes in chemistry and biology. Proton transfer between creatine and surrounding solvent water is underlying the chemical exchange saturation transfer used as a contrast in magnetic resonance imaging. The free energy barrier, determined by first-principles umbrella sampling simulations (E_a^{DFT} 3 kcal/mol) is in the same order of magnitude as the experimentally obtained activation energy. The underlying mechanism is a first proton transfer from the guanidinium group to the water pool, followed by a second transition where a proton is "transferred back" from the nearest water molecule to the deprotonated nitrogen atom of creatine.

1. Introduction

Proton transfer reactions play an important role in many biological systems, especially those which have a large number of hydrogen bonds [1]. The knowledge of exchange rates of labile protons of biological macromolecules provides information about their global and local flexibility [2] as well as access to structural and dynamical information [3–5]. Rate constants can characterize important kinetic processes, such as the opening of base-pairs in nucleic acids [6,7]. Moreover, the chemical exchange is also related to protein folding. For example, the proton exchange rates of native proteins are many orders of magnitude smaller than in unfolded forms [8].

Single-proton-transfer processes are exploited in chemical exchange saturation transfer (CEST) imaging, a new magnetic resonance imaging (MRI) contrast for clinical diagnostics based on the chemical exchange between groups with 'labile' protons in small metabolites and bulk water [9]. Chemical exchange saturation transfer (CEST) enables tissue-structure-weighted and metabolic imaging in biological systems based on chemical exchange properties of small metabolites in normal and tumorous tissues. Saturated protons are transferred from the small solute pool to the huge water pool, producing a measurable attenuation of the water signal intensity.

In this Letter we focus on the small metabolite molecule creatine. Creatine (Cr) is present at high concentrations in tissues and is of primary importance for energy metabolism. The total creatine concentration (creatine and phosphocreatine) in muscles amounts to 45.8 ± 4.8 mM (kg dry weight)$^{-1}$ [10]. Creatine has guanidinium groups, which can exchange protons with water molecules and it produces a CEST contrast in MR imaging *in vivo* in muscle [11] and in multiple-pH creatine model solutions [12]. Creatine CEST contrast *in vivo* can provide interesting insights into muscle energetics and may be used as a tool for diagnosis of muscle diseases [11].

From the CEST experiment the chemical exchange rate between metabolite and water pool can be determined, but not the underlying mechanism and activation energies corresponding to individual steps in the reaction. In the present Letter we study the proton transfer reaction in a creatine-water system by simulations.

We apply umbrella sampling simulations with density functional theory (DFT) to obtain a free energy profile of the proton transfer reaction between creatine guanidinium group and bulk water.

The dependence of chemical exchange rate on temperature and pH for the creatine guanidinium group as well as the effective activation energy $E_{a,eff}^{WEX}$ was determined earlier by water-exchange spectroscopy (WEX) for creatine model-solutions dissolved in phosphate buffered saline (PBS) [12]. We call $E_{a,eff}^{WEX}$ an effective activation energy because this kinetic parameter was determined for creatine in PBS buffer which has different conditions than *in vivo* and is only valid for that particular system. The mechanism of proton transfer reaction has been recently explored by a discrete path sampling approach [13].

2. Computational methods

2.1. Creatine–water system

A zwitterionic creatine structure was surrounded by 93 water molecules in a cubic box of 14 Å side length (Figure 1). The size of the water box was chosen in such a way that it contains enough

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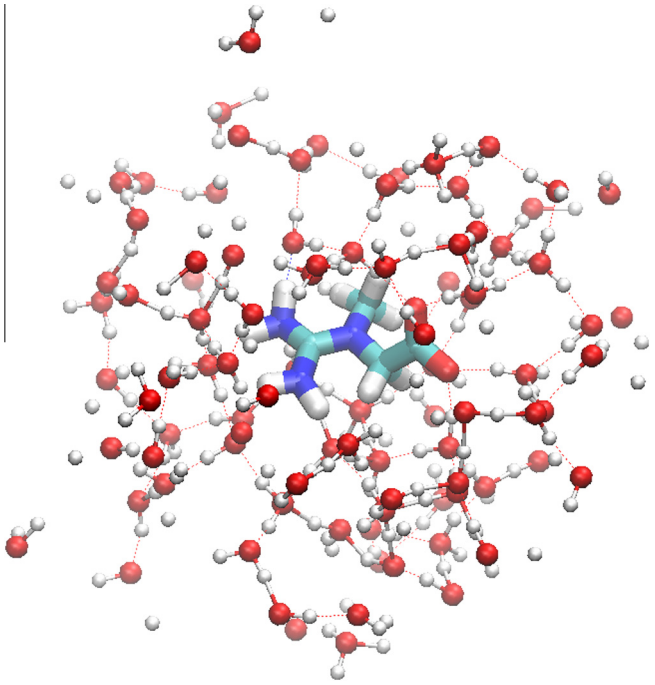

Figure 1. Creatine in zwitterionic form dissolved in a water shell consisting of 93 water molecules.

water molecules to solvate creatine, but remains computationally tractable. The creatine zwitterionic structure is the dominant one at pH = 7.3 according to the CHEBI database [14–17], and also through a pH range of 4–12 [18,19]. We assume that our simulations correspond to this pH range.

2.2. Molecular Dynamics simulations

The first principles Molecular Dynamics (MD) and umbrella sampling simulations applying a DZVP basis set optimized for the Goedecker-Teter-Hutter (GTH), and the BLYP density functional were carried out using the CP2K program [20]. An unbiased production run was performed for a period of 107 ps with 0.5 fs time step in order to relax the solvated creatine-water system. We used a NVT scheme (constant number of particles, volume and temperature) for MD simulations with temperature T = 293 K and periodic boundary conditions, canonical sampling through velocity-rescaling thermostat with every 100 time steps with accepted maximum temperature deviation of 10 K and density cutoff MGRID = 280. The EWALD-type SPME summation for representation of long-range interactions with number of grid points = 25 was used [21,22]. MD trajectories were printed every 1000 steps.

Proton transfer in the creatine–water system constitutes a rare event, which takes place on timescales that are hardly accessible to conventional simulations. Therefore, umbrella sampling is used to simulate this process and to determine the corresponding activation energy. In order to compare the simulations with experimental CEST results we are interested only in the single-proton transfer from the exchangeable group of metabolite to water. Accordingly, the reaction coordinate was chosen as the distance between nitrogen and hydrogen of the creatine guanidinium group: rc_{NH}. This reaction coordinate characterizes the proton transfer from the NH_2 group of creatine to the water-shell and describes the process which occurs in CEST.

The umbrella sampling simulations were performed at the same conditions as the unbiased simulation, but with 1 fs time step and were run for bias potential centered at the different reaction coordinate values subsequently for an initial simulations time of 10 ps. Then, for each umbrella sampling window individually, the simulation was prolonged to 60 ps. The distance distribution for each individually prolonged umbrella sampling window overlaps with the previous and following umbrella sampling simulation windows (see Supplementary). To provide better sampling, umbrella sampling simulation was performed for longer duration (80–170 ps) around the transition state for rc_{NH} = 1.38 Å–1.66 Å. The harmonic potential restrain was imposed on reaction coordinate values rc_{NH} = 1.08 Å to 1.66 Å with a step of 0.02 Å between them. We used 30 windows to describe the proton transfer from the creatine guanidinium group to water. The activation energy which characterizes the height of the barrier of the proton transfer from the guanidinium group of creatine to water was determined by calculating the potential of mean force (PMF) using the weighted histogram analysis method (WHAM) [23,24]. For each simulation window the first 5 *ps* from the umbrella sampling trajectories were not included in the WHAM analyses, as we consider this time as necessary for equilibration around a new reaction coordinate value. The distance between nitrogen and hydrogen of the creatine guanidinium group was extracted at each simulation step for every umbrella window. The WHAM convergence criterium was set to 0.0001 kcal/mol. Moreover, the NH distance is the order parameter along which the kinetic transition network obtained from discrete path sampling simulations can be partitioned into reactant (zwitterionic creatine) and product (deprotonated creatine) region [13].

3. Results and discussion

3.1. Potential of mean force

To understand the proton transfer from the creatine guanidinium group to water, we employed umbrella sampling method. The resulting PMF shows a free energy barrier of the proton transfer from the quanidinium group of creatine to water which amounts to $E_a^{DFT} = 2.44 \pm 0.008$ kcal/mol (or 10.20 ± 0.33 kJ/mol) at T = 293 K (Figure 2, in blue). Note that the error for the free energy given here is the statistical error estimated by bootstrapping. The systematic error of the DFT method is significantly larger (see discussion below).

In addition to the (biased) proton transfer from the creatine guanidinium group to water, another proton transfer is observed from the nearest water proton back to the creatine guanidinium group. This second transfer takes place back and forth several times for reaction coordinates rc_{NH} = 1.36 Å to rc_{NH} = 1.64 Å (Figure 3). The closest water proton is transferred to creatine guanidinium group for the first time at reaction coordinate rc_{NH} = 1.36 Å. At this *rc*-value, the PMF has its highest energy point, which is therefore considered to be the transition state. Having passed that point, the system switches between two states: the deprotonated creatine, which has a proton transferred to water (Figure 3(a)), and the zwitterionic creatine which is formed due to back transfers of the nearest water proton to the creatine guanidinium group (Figure 3(b)). Sampling this second transition (transfer of a water proton) is limited to only a few events, indicating that the free energy barrier directly obtained from the umbrella simulations is underestimated. However, both states are populated in all windows of $rc_{NH} > 1.36$ Å, except for the very last one, before which (at rc_{NH} = 1.64 Å) the primary transfer is already completed. We therefore considered in the traces of the reaction coordinates values only those frames where the back transfer had not occured, i.e. those frames with back transferred proton were not included in the calculation of the PMF using WHAM. As a result a higher value of the proton

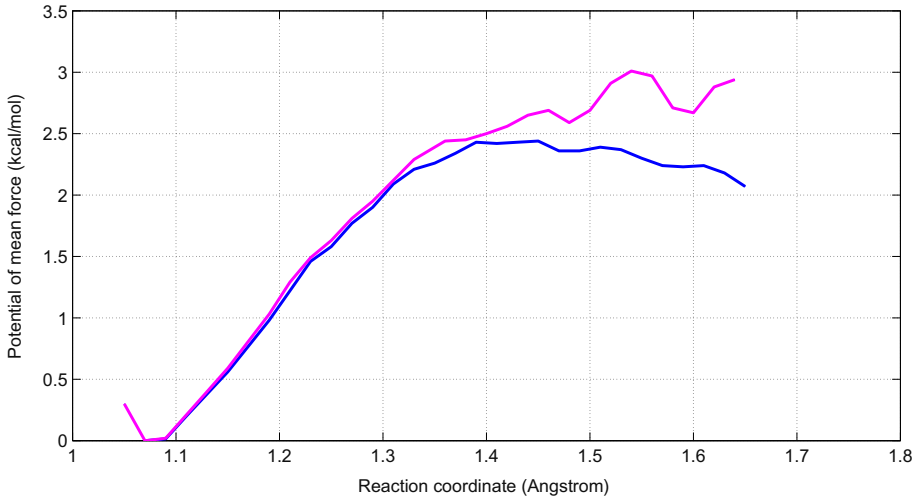

Figure 2. The potential of mean force (PMF) versus the reaction coordinate. The reaction coordinate is the distance between the hydrogen and nitrogen of the guanidinium group of creatine. Blue depicts the PMF obtained from all frames after the first 5 ps. Pink marks the PMF obtained from frames in which no back transfers had occurred. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

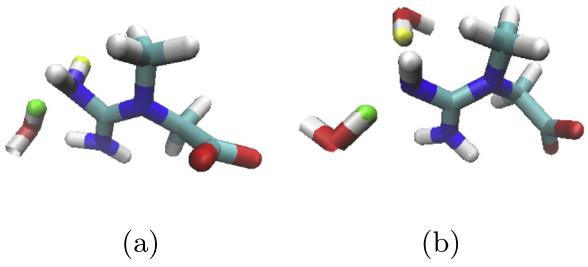

Figure 3. (a) green marks the creatine proton which was transferred to water, b) yellow depicts the water proton which fluctuates between two states: being transferred to the creatine guanidinium group and being in the water-pool ($rc_{NH} = 1.62$Å). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

transfer barrier between the creatine guanidinium group and water was obtained: $E_a^{DFT} = 3.012 \pm 0.007$ kcal/mol (12.60 ± 0.001 kJ/mol), where again the given error range refers to the statistical error.

3.2. Spontaneous proton transfer

This proton transfer barrier between the creatine guanidinium group and water is also rather small, suggesting that a spontaneous proton transfer in the creatine-water system could take place. To check this assumption, the creatine-water structure from umbrella sampling simulation was considered and free, unbiased, MD was run for 348 ps and analysed for the spontaneous proton transfers. From this free MD, the following traces were plotted: the distance trace between hydrogen and nitrogen of the guanidinium group (rc_{NH}) over the entire simulation time (Figure 4a) and the distance trace between the hydrogen atom of the creatine guanidinium group and the closest water oxygen atom (Figure 4b).

The trace of the distance between the closest water oxygen atom and the hydrogen atom of the guanidinium group of creatine shows that the smallest distance between these two atoms is 1.18–1.19 Å in four free MD snapshots (Figure 4b). This is a loose bond because it lasts only 0.004 ps (Figure 5)). This observation indicates that a spontaneous proton transfer from the creatine guanidinium group to water can indeed occur during the free MD simulation albeit with only low probability. From the probability ratio, i.e. the ratio of the number of frames, between the transferred state with deprotonated creatine and the zwitterionic creatine, a free energy difference of 11 kT, i.e. ≈ 7 kcal/mol is estimated. Of course this estimate can at best give an idea of the order of magnitude since a single event is statistically not meaningful. However, the unbiased MD simulation shows that, in general, a spontaneous proton transfer is possible as the low barrier computed from the umbrella sampling simulation suggests.

3.3. Proton transfer pathway

From the umbrella sampling simulation of creatine in a water-shell, we obtained the following proton transfer pathway from the guanidinium group of creatine to water: zwitterionic creatine (Figure 6(a)) forms a bond with the nearest water molecule, as a result the creatine guanidinium proton is shared between the nearest water proton and guanidinium group of creatine (Figure 6(b)), then the guanidinium proton is transferred to the creatine water-shell (Figure 6(c)).

3.4. Comparison with experiment

The proton transfer (PT) barrier from the creatine guanidinium group to water calculated from BLYP umbrella sampling simulations is $E_a^{DFT} = 3.012 \pm 0.007$ kcal/mol (12.60 ± 0.001 kJ/mol).

The dependence on pH and temperature of chemical exchange rate was measured for model-solutions of creatine dissolved in PBS buffer by Goerke [25,12] using water exchange spectroscopy (WEX). WEX is an inverse CEST experiment: labelled water magnetization is transferred to the metabolite pool during the mixing time T_m as a result of chemical exchange between the metabolite and water pools [26]. The transfer properties of the labelled water longitudinal magnetization is detected on the metabolite pool and is used to estimate the metabolite-water proton transfer rates [26]. The WEX method allows to determine kinetic parameters, such as activation energy of the guanidinium proton transfer to water and collusion frequency. The effective activation energy of the proton transfer from the guanidinium group of creatine to water amounts to $E_{a,eff}^{WEX} = 7.71 \pm 1.77$ kcal/mol (or 32.27 ± 7.43 kJ/mol) [12].

The value for the free energy barrier obtained from umbrella sampling simulation ($E_a^{DFT} = 3.012 \pm 0.007$ kcal/mol) is significantly

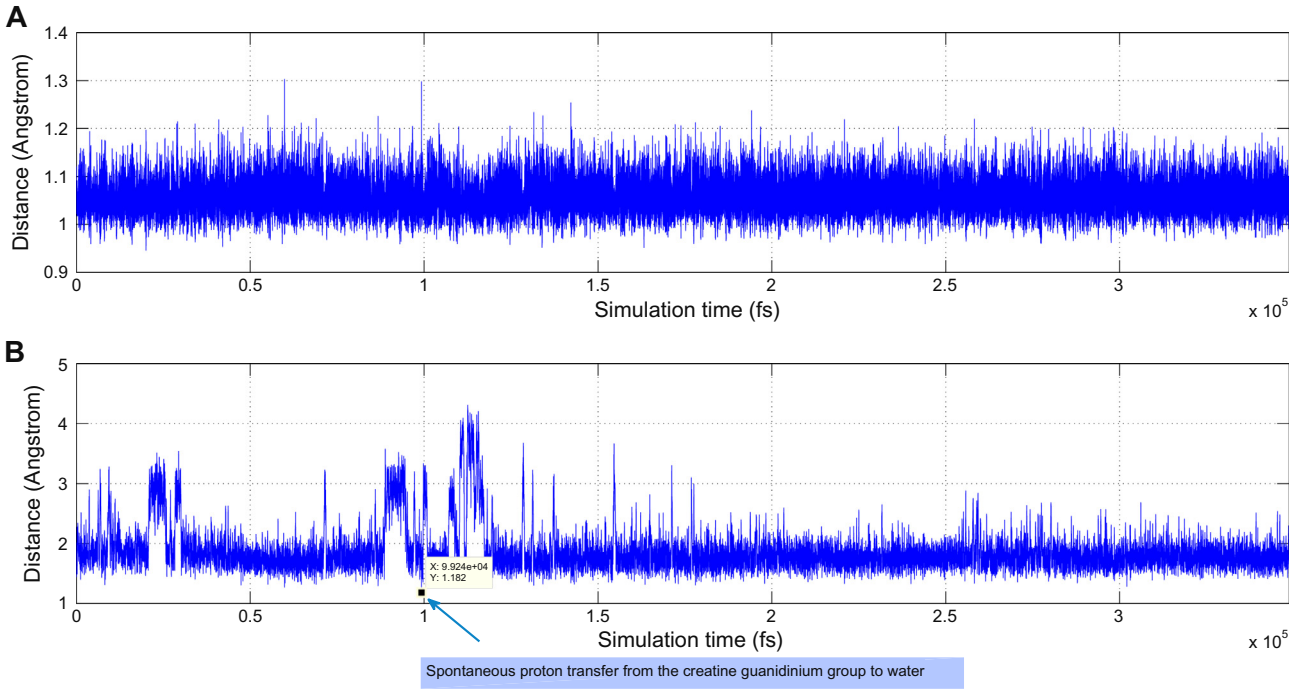

Figure 4. (a) trace from the 348 ps free molecular dynamics simulations of the distance between hydrogen and nitrogen of the guanidinium group of creatine. (b) trace between the hydrogen of the guanidinium group of creatine and the oxygen of the nearest water molecule.The arrow indicates the position in the trace where the spontaneous proton transfer from the creatine guanidinium group to water has occurred.

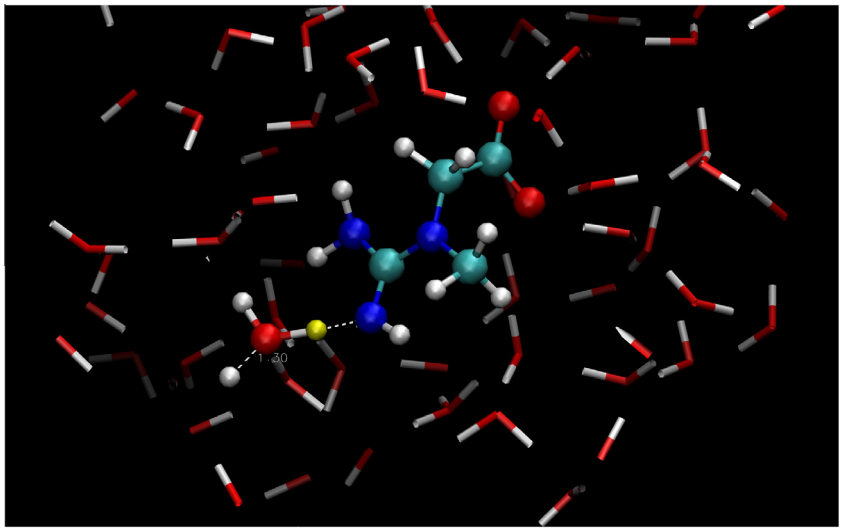

Figure 5. Spontaneous proton transfer from the creatine guanidinium group to water which occurs at 99 ps during 348 ps free MD. The "mobile" proton of the guanidinium group of creatine is marked in yellow. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

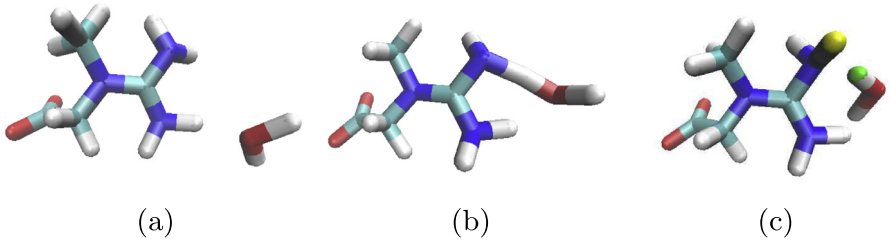

Figure 6. Proton transfer mechanism in creatine-water system studied by umbrella sampling simulations: (a) the zwitterionic form of creatine, $rc_{NH} = 1.12$ Å, (b) the zwitterionic creatine is forming a bond with the closest water molecule, $rc_{NH} = 1.48$ Å, (c) deprotonated creatine with back transferred proton. The proton of creatine (green) was transferred to water-pool, $rc_{NH} = 1.66$ Å. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

smaller than the activation energy estimated from the WEX experiment.

According to Sadhukhan [27], BLYP underestimates the barrier by 2 kcal/mol. The proton transfer barrier for the guanidinium group of creatine is probably 2 kcal larger than the value obtained from umbrella sampling simulations. Taken into account this systematic error the proton transfer barrier from the guanidinium group of creatine to the water-shell calculated from BLYP umbrella sampling is in the same order of magnitude as the value obtained by WEX experiments.

4. Conclusion

In this Letter we investigated the proton transfer from creatine guanidinium group to bulk water by umbrella sampling simulations using BLYP. The pathway observed in the simulations is a single proton transfer from the guanidinium group of creatine to the water pool, followed by back protonation from another, nearby water molecule to creatine. The activation energy of proton transfer in creatine water obtained from the free energy profile $E_a^{\mathrm{DFT}} \approx 3$ kcal/mol is lower than the value $E_{\mathrm{a,eff}}^{\mathrm{WEX}} \approx 7.7$ kcal/mol derived from WEX experiments. Taking into account a systematic error of $\pm$ 2 kcal/mol for simulations using BLYP, our results show an acceptable agreement with the outcome of WEX experiments.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.cplett.2014.03.045.

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