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On the Utilization of Energy Minimization to the Study of Ion Selectivity

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ABSTRACT The major pitfalls in studying ion selectivity in binding site models using energy minimization based methods are examined and discussed. It is shown that the properties derived from energy minimization are strongly configuration-dependent and that the results should be interpreted with caution. It is concluded that computational studies of ion selectivity must include thermal fluctuations and entropic effects.

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Two main factors limit computational studies aimed at characterizing ion selectivity in biological channels: 1), the accuracy of the microscopic model underlying the potential energy surface, and 2), the reliability of the computational procedure designed to account for thermal fluctuations. Striking a balance between accuracy and sampling is challenging, but necessary to draw meaningful conclusions. At one extreme, it is possible to achieve extensive statistical sampling with simplified models, albeit at the risk of representing microscopic interactions by relatively crude approximations. At the other extreme, if those interactions are generated from sophisticated quantum mechanical (QM) approaches, then achieving a meaningful sampling of the relevant configurations can become computationally prohibitive. For this reason, QM approaches are often used in combination with energy minimization procedures to characterize relatively small atomic systems. The problems with energy minimization are seriously aggravated in the case of large systems, where the energy landscape becomes extremely rugged. It is the purpose of this article to discuss the pitfalls associated with a strategy based on energy minimization, and to highlight the importance of thermal fluctuations and entropic effects in computational studies of ion selectivity.

In a number of QM studies of ion selectivity, only a single ‘‘optimal’’ energy-minimized configuration of the system is considered, and fluctuations around this configuration are either ignored (1) or incorporated via a harmonic vibrational approximation (2,3). Such a strategy encounters difficulties if there are multiple local energy minima. To illustrate the nature of the problem, we compare the results from energy minimization with those from free energy perturbation molecular dynamics (FEP/MD) simulations for the same atomic system. For the sake of simplicity, we first consider a reduced model of ion coordination consisting of four to eight water molecules surrounding one cation (K^+ or Na^+) confined with a steep potential within 3.5 Å (4,5). Both the nonpolarizable CHARMM force field (6) and the recently developed Drude-based polarizable (7,8) models were used. The Drude polarizable models compare favorably with the results from the QM calculations (8). Langevin dynamics simulations were performed for 50 ps after equilibrated for 50 ps at 298.15 K, and 500 instantaneous configurations (one every 0.1 ps) were saved for analysis. The saved configurations were extensively minimized for 5000 steps with a steepest descent algorithm. To provide a standard comparison, FEP/MD simulations were performed for the same system for 1 ns to estimate $\Delta G^{\mathrm{site}}_{\mathrm{K,\,Na}} = G^{\mathrm{site}}_{\mathrm{Na}} - G^{\mathrm{site}}_{\mathrm{K}}$ (4,5).

As observed in Fig. 1 *a* and *c*, the energy of the local minima can differ by as much as 4 kcal/mol for K^+ and 5 kcal/mol for Na^+ for a simple system with one ion and eight water molecules. The distribution resulting from randomly picking one possible optimized cluster for each system is shown in Fig. 2. The energy difference between uncorrelated minimized configurations with K^+ and Na^+ shows a broad distribution, with a standard deviation of as much as 2 kcal/mol. This is consistent with the finding by Varma and Rempe that there exists a significant effect on energetics due to anharmonicity and configurational fluctuations in clusters of more than five water molecules (3). The considerable uncertainty in the relative energy difference is due to the large uncorrelated variations between the energy of the multiple local minima of the clusters with K^+ or Na^+ modeled separately. Alternatively, rather than assuming that the energy minimization gets trapped in one local minimum, one might attempt to cure this problem by using minimization algorithms, guaranteeing that the absolute ‘‘best’’ global minimum is discovered. As shown in Fig. 3, selecting only the best minimized structures from the optimized configurations actually gives rise to a systematic error in estimating the energy difference between K^+ and Na^+. The error for the system of eight water molecules is as large as 3 kcal/mol, which is significant for studying K^+-selectivity in such a small cluster. These types of problems are possibly reflected in the study of Varma and Rempe (3), which is based on energy minimization of an ion in a small water cluster. Even

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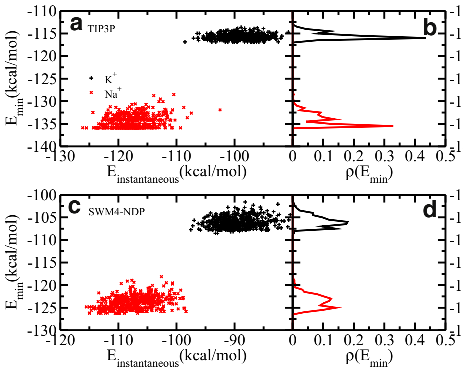

FIGURE 1 Energy distribution of minimized structures of eight water and one cation (K^+ or Na^+) system sampled from MD simulations. (*a*) The energies of the minimized structures versus the energies of the corresponding MD sampled structures with TIP3P model; (*b*) The distribution of the energies of the minimized structures with TIP3P model; (*c*) Same as *a* for SWM4-NDP model; and (*d*) same as *b* for SWM4-NDP model.

though the adopted density functional theory gives accurate results for water-cation dimer interaction, the estimated bulk hydration free energy difference between K^+ and Na^+ is -21.5 ($-93.3 + 71.8$) kcal/mol (3), deviating by ~4 kcal/mol compared to the experimental value of -17.2 kcal/mol. The continuum dielectric treatment of long-range electrostatic may be, in part, responsible for the discrepancy as well (3). Test calculations similar to those shown in Fig. 2 for a reduced system comprising of one ion and eight *N*-methylacetamide molecules, which is often used to mimic the binding site S2 of the KcsA channel, give a standard deviation ~2.2 kcal/mol.

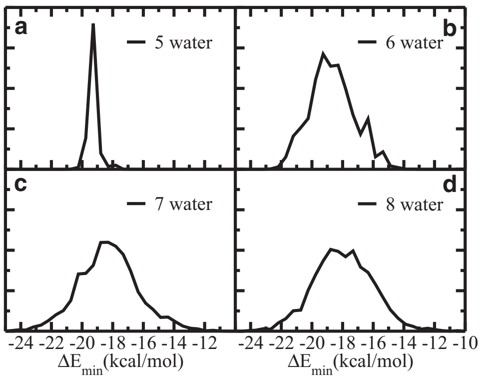

FIGURE 2 Energy difference distribution between Na^+ and K^+ in a cluster of five to eight water molecules estimated by repeated energy minimizations with SWM4-NDP polarizable water model. The energy difference is calculated by randomly sampled the data presented in Fig. 1 for 5000 times. (*a*) The average of the energy ($\langle \Delta E_{\min} \rangle$) difference is -19.0 kcal/mol with a standard deviation ($\sigma(E_{\min})$) of 0.3 kcal/mol; (*b*) $\langle \Delta E_{\min} \rangle = -18.4$ kcal/mol, $\sigma(\Delta E_{\min}) = 1.5$ kcal/mol; (*c*) $\langle \Delta E_{\min} \rangle = -18.0$ kcal/mol, $\sigma(\Delta E_{\min}) = 1.9$ kcal/mol; and (*d*) $\langle \Delta E_{\min} \rangle = -17.7$ kcal/mol, $\sigma(\Delta E_{\min}) = 2.0$ kcal/mol.

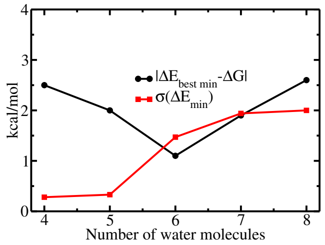

FIGURE 3 Difference between the free energy difference based on FEP (ΔG) and the energy difference based on comparing the global minima, compared with the standard deviation estimated by the data shown in Fig. 2.

The problems observed with the small systems comprising an ion and a few water molecules get considerably worse when a large system is considered. To illustrate the problem with energy minimization in large biomolecular systems, we consider a model of the KcsA channel embedded in a DPPC membrane as in Noskov et al. (4). A similar system was studied by Miloshevsky and Jordan using energy minimization (9). Here, 100 instantaneous configurations were extracted from molecular dynamics (MD) simulation, with either K^+ (KcsA$\cdot K^+$) or Na^+ (KcsA$\cdot Na^+$) bound in the S2 binding site and extensive energy minimizations were performed for each configuration. As shown in Fig. 4, very large variations arise for the energy difference between K^+-bound and Na^+-bound initiated from the same snapshot. The energy difference strongly depends on the particular configuration comprising the protein, the membrane, the water, and the ion used to start the energy minimization. When the energy minimization is initiated from a KcsA$\cdot K^+$ snapshot, the average total energy difference is -11 kcal/mol. Thus, the S2 binding site appears selective for K^+ over Na^+ by 7 kcal/mol, which compares relatively well with 5.3 kcal/mol from FEP/MD calculations (4). However, when the energy minimization is initiated from a KcsA$\cdot Na^+$ snapshot, the average total energy difference is ~-25 kcal/mol and now the energy difference indicates that the site is selective for Na^+ over K^+ by 7 kcal/mol. The erroneously result arises because the channel and its selectivity filter are trapped in the local energy well corresponding to the configurations with a bound Na^+. The fluctuations away from the starting configuration are ignored, yielding a spurious preference for Na^+.

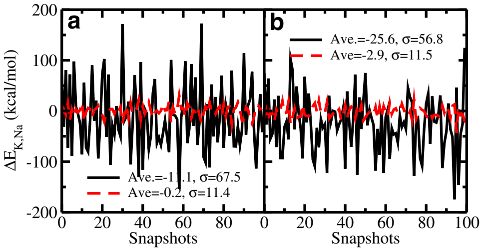

FIGURE 4 (*a*) Total energy (*black*) and bonded component (*red*) energy differences (in kcal/mol) between the minimized structures with K^+ bound or Na^+ bound with a structure sampled in KcsA$\cdot K^+$. (*b*) Same as *a* sampled in KcsA$\cdot Na^+$. In all cases, minimization was carried out until the average gradient was <0.001 kcal/(mol Å) comprising 500 step steepest descent and at least 2000 step adopted basis Newton-Raphson.

An energy minimization strategy is clearly associated with a very large uncertainty and a high dependence upon the starting configuration. Likewise, the decomposition of the energy difference into specific contributions is also uncertain. When the energy minimization is initiated from a $\mathrm{KcsA \cdot K^+}$ snapshot, the bonded term does not contribute to the total energy difference and ion selectivity. But when it is initiated from a $\mathrm{KcsA \cdot Na^+}$ snapshot, the bonded term contributes ~3 kcal/mol to the 7 kcal/mol selectivity favoring $\mathrm{Na^+}$. This observation casts doubt on the analysis of Miloshevsky and Jordan (9) that the ''topological'' forces play a dominant role in $\mathrm{K^+}$ selectivity based on differences of ~3 kcal/mol from the bonded energy term favoring $\mathrm{K^+}$.

The use of energy minimization is frequently justified by noting that the entropic contribution to the free energy difference between $\mathrm{K^+}$ and $\mathrm{Na^+}$ in water or the channel nearly cancels out (9). However, the implication of entropy cancellation is misconstrued in this argument. Although the relative free energy of ions is indeed dominated by enthalpy and not by entropy (10,11), this does not imply that the absolute effect of thermal fluctuations can be ignored. In flexible systems, selectivity cannot be understood on the basis of a few energy-minimized configurations because the free energy differences result from a thermal averaging over an ensemble of accessible configurations (11,12). Obviously, the channel is complex and, in the absence of a proper thermal averaging, unreliably large variations from all energy terms are observed depending upon the starting configuration.

In summary, the analysis presented here indicates that the studies of ion selectivity based on energy minimization must be interpreted with great caution for these anharmonic systems. Even in the case of a system as simple as a single ion coordinated by a small number of water or *N*-methylacetamide molecules, proper accounting for thermal fluctuations appears to be critical for obtaining reliable estimates of free energy differences. The problems become much worse in the case of large complex biomolecular structures. Here, there is no obvious justification for energy minimization since there are well-established alternative computational strategies. For example, FEP/MD simulations offer a robust computational approach to extract the proper thermodynamic quantities needed to characterize ion selectivity. Although the all-atom force fields used in FEP/MD are approximations with well known limitations (13), the results for a number of biomolecular systems are in good agreement with experimental estimates. This shows that the current FEP/MD methodology based on empirical force fields can be sufficiently accurate to provide mechanistic insights about ion selectivity (4,5,11,14,15). On this note, it may be important to recall that, although the notion of a ''first-principles'' representation of the microscopic forces is an attractive aspect of QM approaches, even simple models can sometimes yield meaningful results, as long as they are physically sound and they are properly sampled. For example, a highly idealized model of $\mathrm{Ca^{2+}}$ channels accounting for energy and particle fluctuations has been able to reproduce much of the experimentally observed behavior under a wide range of conditions (16,17,18).

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