we may find cases where it is necessary to include multiple-step binding. This can be included into our model just as any additional intermediate nucleotide state in the cycle of ATP hydrolysis can be included should we wish to simulate a particular event. The inclusion of an additional binding step is not the only difference between our models. The differences are summarized in the legend to Figure 1.

The point was made that tropomyosin should be treated as a continuous cable, but the Hill model assumed that a single tropomyosin covering seven actin monomers acts as a unit. In the M and G model, the size of the cooperative unit changes with conditions. Tobacman and Butters (2000) have incorporated a very large degree of flexibility into their model by allowing each actin monomer to be treated independently. In the Hill model, the cooperativity is altered by the strength of the interaction between adjacent tropomyosin molecules (the parameter *Y*). It is also possible to make the size of the cooperative unit variable in the Hill model while still preserving the more fundamental differences with the M and G model. It is mathematically nontrivial to rigorously incorporate this flexibility into either the Hill model or the M and G model. Because this level of detail was not necessary to simulate the regulation of ATPase activity, it was not incorporated into our model. We must not lose sight of the fact that this is a model.

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On the Potential Functions used in Molecular Dynamics Simulations of Ion Channels

The determination of the structure of the KcsA K^+ channel represents an extraordinary opportunity for understanding biological ion channels at the atomic level. In principle, molecular dynamics (MD) simulations based on detailed atomic models can complement the experimental data and help to characterize the microscopic factors that ultimately determine the permeation of ions through KcsA. A number of MD studies, broadly aimed at analyzing the dynamical motions of water molecules and ions in the KcsA channel, have now been reported (Guidoni et al., 1999; Allen et al., 1999; Shrivastava and Sansom, 2000; Åqvist and Luzhkov, 2000; Bernèche and Roux, 2000; Biggin et al., 2001; Luzhkov and Åqvist, 2001; Crouzy et al., 2001). The potential functions that were used to calculate the microscopic interatomic forces and generate the dynamical trajectory are

Submitted August 30, 2001, and accepted for publication December 11, 2001.

0006-3495/02/03/1680/08 $2.00

TABLE 1 Potential energy function and MD simulations of KcsA

MD Simulations	Protein and Lipid	Type of Force Field	Water	Ions
Guidoni et al. (1999)	AMBER*	All atoms	TIP3[†]	Åqvist (1990)
Bernèche and Roux (2000)	CHARMM PARAM22[‡]	All atoms	TIP3[†]	Beglov and Roux (1994)
Allen et al. (1999)	CHARMM PARAM19[§]	Extended atoms	ST2[¶]	Heinzinger (1985)
Shrivastava and Sansom (2000)	GROMOS[‖]	Extended atoms	SPC**	Straatsma et al. (1988)
Åqvist and Luzhkov (2000)	GROMOS[‖]	Extended atoms	SPC**	Åqvist (1990)

*Cornell et al. (1995).
[†]Jorgensen et al. (1983).
[‡]Schlenkrich et al. (1996) for lipids and MacKerell et al. (1998) for proteins.
[§]Brooks et al. (1983).
[¶]Stillinger and Rahman (1974).
[‖]Hermans et al. (1984).
**Berendsen et al. (1981).

listed in Table 1, where they can be seen to differ significantly. In particular, the atomic partial charges and the Lennard–Jones radii, which are at the heart of the potential function, varied widely. Furthermore, some include all atoms (AMBER and CHARMM PARAM22), whereas others are extended-atom models that treat only the polar hydrogens able to form hydrogen bonds explicitly (CHARMM PARAM19 and GROMOS). How these differences affect the results of MD calculations is an important concern of all scientists involved in investigations of ion channels, theoreticians and experimentalists alike. It is the goal of this short letter to discuss important aspects of potential functions related to MD studies of ion permeation.

For meaningful theoretical studies of permeation, it is necessary to have a potential energy function providing a realistic and accurate representation of the microscopic interactions. In practice, this presents a difficult challenge. The permeation process through KcsA involves the partial dehydration of a K^+ ion, followed by the translocation through the interior of a narrow pore of 12-Å-long, lined by backbone carbonyl oxygens, which acts as a selectivity filter (Doyle et al., 1998). Thus, the conductance and selectivity of the KcsA channel results from a delicate balance of very strong microsopic interactions, the large energetic loss of dehydration being roughly compensated by coordination with main chain carbonyl oxygens. Gas phase experiments on model systems provide the most direct information concerning the individual microscopic interactions (Džidić and Kebarle, 1970; Klassen et al., 1996). High-level quantum-mechanical ab initio calculations can also be used to supplement the (often scarce) information available from experiments (Roux and Karplus, 1995). The interaction of ions with a single water molecule, or with a single isolated *N*-methylacetamide (NMA) molecule, an excellent model of the backbone carbonyl of proteins, is of particular interest.

The most important microscopic interactions energies for ion permeation through the K^+ channel are given in Table 2. Despite the considerable uncertainty in the experimental data and the ab initio calculations, both clearly indicate that the interaction of cations with a single NMA is substantially larger than with a single water molecule. The binding enthalpy of K^+ with a water molecule is 17.9 kcal/mole, whereas it is roughly 25–30 kcal/mole with NMA. The interactions are even larger in the case of Na^+. This general trend is generally reproduced by all the potential functions, with the exception of GROMOS (Hermans et al., 1984). In this case, the interaction of K^+ and Na^+ with a single NMA is actually smaller than the interaction with a single water molecule. The difference in the interaction energy is directly related to the atomic charges assigned to the peptide backbone, i.e., the atomic charges from GROMOS (Hermans et al., 1984) are about 60% to 75% relative to those from AMBER (Cornell et al., 1995), CHARMM PARAM19

TABLE 2 Microscopic interactions (kcal/mol)*

K^+		Na^+		
Water	NMA	Water	NMA	Reference
17.9	28.3–32.3	24.0	33.7–39.0	Gas phase exp (Džidić and Kebarle, 1970; Klassen et al., 1996)
15.9–17.6	24.8–31.7	24.0–25.8	38.4–40.4	Ab initio (Roux and Karplus, 1995)
18.2	23.7	23.2	29.5	Guidoni et al. (1999)
18.9	24.1	25.5	30.1	Bernèche and Roux (2000)
18.3	21.9	24.8	27.9	Allen et al. (1999)
17.8	16.6	22.8	20.6	Åqvist and Luzhkov (2000)
17.6	16.8	26.3	23.6	Shrivastava and Sansom (2000)

*The interactions energies based on the different force fields were calculated by us assuming a rigid geometry of the water or NMA molecule. When unavailable for a given potential function, the atomic partial charges of NMA were deduced from those of a glycine dipeptide.

(Brooks et al., 1983), or CHARMM PARAM22 (MacKerell et al., 1998).

Conductance and selectivity are primarily governed by relative free energies. For this reason, it is essential to consider also thermodynamics properties in the parametrization of the potential function in addition to the microscopic interactions. The solvation free energy of cations in liquid water and liquid NMA are particularly important for calibrating a potential function. In the case of water, it is possible to reproduce both the microsopic interactions and the solvation free energy of ions with the current potential functions (Straatsma et al., 1988; Åqvist, 1990; Beglov and Roux, 1994). For example, the solvation free energy of K^+ in liquid water is ~80 kcal/mol (Dorman et al., 1996) (though there is considerable uncertainty, see Pliego and Riveros (2000)). Such a value can be reproduced quite well with a potential function yielding a microsopic interaction with a single water molecule on the order of 17–18 kcal/mol (Straatsma et al., 1988; Åqvist, 1990; Beglov and Roux, 1994). In contrast, MD free energy calculations indicate that it is very difficult to reproduce both the cation–NMA microscopic energy and the solvation free energy in liquid NMA with current biomolecular potential functions. For example, the CHARMM PARAM22 potential function, which gives an interaction energy of 24.1 kcal/mol with a single NMA, yields a free energy of ~88 kcal/mol in liquid NMA (S. Bernèche and B. Roux, unpublished results). Although the solvation free energy of K^+ in liquid NMA is not known experimentally, data from other liquid amides suggests that such a large value is unrealistic and that a reasonable estimate should be ~80−82 kcal/mol (Cox et al., 1974).

For a given potential function, the calculated ion solvation free energy in liquid NMA is expected to be reflected directly upon the stability of K^+ in the selectivity filter during MD simulations of the KcsA channel. Therefore, the present analysis suggests that the K^+ ions bind too strongly to KcsA by ~5–10 kcal/mol in MD simulations based on the all-atoms potential function AMBER and CHARMM PARAM22, such as used by Guidoni et al. (1999) and Bernèche and Roux (2000), respectively. In contrast, because the microscopic interaction energy of K^+ with a single NMA is only on the order of 16–17 kcal/mol (see Table 2), the K^+ ions bind probably too weakly to KcsA by as much as 20 kcal/mol in MD simulations based on the extended-atom GROMOS potential function such as used by Åqvist (Åqvist and Luzhkov, 2000; Luzhkov and Åqvist, 2001) and Sansom (Shrivastava and Sansom, 2000; Biggin et al., 2001). To obtain a free energy of ~80 kcal/mol in liquid NMA, one can adjust the Lennard–Jones parameters of the cation–carbonyl oxygen pairs and reduce the microscopic cation–NMA interaction energy to ~21.6 kcal/mol (S. Bernèche and B. Roux, unpublished results). This is one way to parametrize and calibrate the potential function for theoretical studies of ion permeation through KcsA.

Clearly, if the potential function was an exact representation of the Born–Oppenheimer energy surface, success in reproducing the microsopic interactions would automatically lead to accurate thermodynamic properties. But current biomolecular potential functions try to account for many-body polarization effects in an average way using an effective parametrization of the atomic partial charges. Because of this approximation, the optimal parametrization is the result of a compromise between an accurate representation of the microscopic energies and bulk solvation properties. We believe that such potential functions can yield meaningful results of semi-quantitative accuracy. Recently, we have taken these factors into consideration in calibrating the potential function for a calculation of the free energy surface governing conduction of K^+ ions through the selectivity filter of the KcsA K^+ channel (Bernèche and Roux, 2001). In the particular case of this study, it should be stressed that meaningful results were not obtained until the potential function was adjusted to reproduce the correct free energies of K^+ in liquid water and liquid NMA. In general, it ought to be possible to calibrate any potential function to reproduce solvation free energies using a similar approach (though the significantly underestimated ion–NMA interaction energy based on the GROMOS force field might require some modifications of the atomic charges). Further analysis suggest that the situation might be more difficult in the case of a small cation such as Na^+ (Roux, 1993), suggesting that a quantitative simulation of the microscopic factors governing ion selectivity is probably beyond the ability of current biomolecular potential function.

Ultimately, the influence of nonadditive many-body polarization should be viewed in a wider perspective. At the present time, computational chemists and theoreticians are actively pursuing the development of a new generation of force fields that will include induced polarization for computational studies of biological systems (Halgren and Damm, 2001). But much more work is needed before such potential functions are ready to be used in simulations of biological ion channels. Meanwhile, we believe that MD studies of ion channels can still yield meaningful results, as long as they are based on effective potential functions that have been calibrated to correctly reproduce solvation free energies.

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