Letters to the Editor

Modeling Thin Filament Cooperativity

We were very interested in the paper by Chen et al. (2001) on the modeling of the kinetic and equilibrium binding of myosin S1 to regulated actin filaments, containing actin, tropomyosin, and troponin (ATmTn). This is a formidable task, and the authors of the paper are to be commended on their considerable achievement. They have made a detailed comparison of the Hill et al. two-state model (1980) (referred to as the Hill model) and the McKillop and Geeves three-state model (1993) (referred to as the M and G model) and concluded that both can adequately describe the data. This could be interpreted, using Occam's razor, that a three-state model is not necessary. Although we would not wish to disagree with their calculations, we wish to point out that the authors: 1) have considered only some of the available data to test the two models; 2) have not compared the ability of the models to address fundamental issues in thin filament regulation; and 3) have not related the mathematical parameters of the models to the properties of the components.

We believe that the M and G model is a more useful model compared with the Hill model because: 1) the three states of ATmTn (Blocked-Closed-Open (M)) can be more readily related to three positions of Tm observed on actin, although the model was developed independently of structural information. 2) The two-step binding of myosin to actin can easily be integrated into the three states, including the coupling between the isomerization step and the C-O equilibrium. 3) It is more readily testable because the parameters that are used can be directly related to the properties of Tm, the regulatory component (such as strength of end-to-end interactions and flexibility which depend on amino acid sequence), and the modification of Tm function by Tn and Ca^{2+}, the allosteric components of the thin filament. 4) The M and G model is a complete biochemical model that involves equilibria between states that are affected by Ca^{2+} and myosin, rather than states that are defined by the absence or presence of Ca^{2+} or myosin. A given state, therefore, may not be fully occupied under a given set of experimental conditions (Table 1). 5) The M and G model can explain a much larger set of data, which were not considered in the Chen et al. (2001) paper.

These issues are expanded upon as follows:

The properties of the two- and three-states must be defined. Chen et al. described the Hill model as having two states, each with three substates (0, 1, and 2 Ca^{2+} bound for a total of six states). The M and G model on the other hand is described as a three-state model. Unless the meaning of the states is defined, the precise number of biochemical states will not be clear. If the states refer to the ATPase-activating potential of the thin filament then our model, like the Hill model, is a two-state model with the ATPase either *off* or *on*, and the ATPase *off* state consists of two substates, B and C (Table 1). The three-states of actinTmTn in our model, however, are defined in terms of three distinct myosin-binding properties of the actinTmTn complex. These binding states have more recently been associated with three distinct locations of Tm on the actin surface (Vibert et al., 1997; Holmes, 1995) as originally postulated by McKillop and Geeves (1993) and by Lehrer and Morris (1982).

The fundamental problem with any two-state model is that it does not readily take into account the major structural change occurring on removal of Ca^{2+} (fiber x-ray scattering (Holmes, 1995), electron microscopy (Vibert et al., 1997; Lehman et al., 2000), fluorescence probes (Bacchiocchi and Lehrer, 2000)), which has been interpreted as a large movement of Tm over the surface of actin away from a site where it blocks most of the myosin head-binding site on actin to a site where there is little direct interference. Chen et al. indicate that there are sufficient substates to account for the structural data. To make this argument the properties of the two fundamental states and the substates need to be defined. If the properties of the substates vary, then it is no longer a two-state equilibrium model. The assumed properties of the three states of the M and G model (*B*, *C*, and *M*) were carefully defined in 1993 and the evidence produced since that time has not required any change in these definitions. Furthermore, the model is readily compatible with the structural and spectroscopic data. The fundamental issue here is that all equilibrium studies require that actinTm and actinTmTn ($+Ca^{2+}$) exist (to ~80%) in a state which does not readily bind myosin (our *C*, or closed state). Removal of calcium turns the system more completely "off" and the issue is whether this simply changes the equilibrium between the two states (as in the original Hill et al. model) or indicates the presence of a new state, B, with different

TABLE 1 Properties and occupancy of the three thin filament states of McKillop and Geeves

Properties	B	C	O (M)	
Myosin-binding ability	none	weak (A)	strong (R)	
ATPase	OFF	OFF	ON	
Composition	Occupancy			
	B	C	O (M)	Tm position
−Tn	0	0.8	0.2	inner/outer
+Tn−Ca	0.7	0.25	0.05	outer domain
+Tn+Ca	0	0.8	0.2	inner/outer
+myosin	0	0	1.0	inner domain

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properties. Our data and the structural data point to a new state with different properties independent of whether the new state is called a state or substate.

The importance of the two-step myosin binding. In all our published work from the original version of the model (Geeves and Halsall, 1987) to the most recent comparison with Hill (Maytum et al, 1999) model we acknowledged that the two models were equivalent in describing equilibrium myosin-binding data. Our model was originally proposed (Geeves and Halsall, 1987) as an alternative to the Hill model because of the new insights that came from the multistep docking of myosin S1 to actin. The role of K_2, the equilibrium constant for the so-called A-to-R isomerization of myosin when bound to actin, is central to the underlying mechanism and can be defined independently of the model of regulation. This parameter is very powerful in predicting the ability of any myosin nucleotide complex to activate the thin filament. Chen et al. makes no use of this information.

Chen et al. did not use all the available data for the argument. The original evidence for the 3rd, *B* state, was supported by both kinetic and equilibrium myosin-binding data which included equilibrium titrations in which K_2 was varied from a value of 3 to 200. Kinetic binding data included measurements where the rates of S1 binding to actin were varied fourfold by varying the concentrations of the proteins and reduced sixfold by the use of a π analog bound to the nucleotide pocket. All the data can be well described by our model.

Chen et al. state "Nor has it been clearly demonstrated that the Geeves model is really able to predict the characteristic family of time courses of S1 binding for different S1 and actin concentration." We assume that this is referring to the kinetic data with S1 in excess, because all data with actin excess can be perfectly fitted without recourse to the complex modeling of Chen et al. We have also presented a range of data in 1995 for the kinetics of excess S1 binding to actin (Head et al., 1995). We used a very simple kinetic model and demonstrated that the lag phases observed were compatible with our model not simply for data with and without calcium but for a range of calcium concentrations.

Evidence that the B *to* C *states are in true equilibrium.* Head et al. (1995) tested the properties of the *B* state and the effect of several parameters on the equilibrium constant K_B were assessed. These included variations of actin concentration (fivefold), temperature (5–40°C), ionic strength (0.01–0.4 M), and calcium concentration (pCa 4–9). In all cases, the data were compatible with an equilibrium between the *B* and *C* conformations as predicted in the original model.

The most significant experiment involved the variation of Ca^{2+} concentration. The data showed that K_B was Ca^{2+}-sensitive and the calcium dependence of K_B showed a Hill coefficient of 1.8 and a midpoint at pCa 5.6; data very similar to the behavior of thin filaments in vitro and in muscle fibers (Potter and Gergely, 1975, Grabearek et al., 1983). Furthermore, similar results were produced using cardiac Tn with the predicted reduced Hill coefficient and a phosphorylation dependent shift in the midpoint of the curve (Reiffert et al., 1996; Zang et al., 1995). Significantly, the calcium dependence of K_B is the only factor required to produce a fit to the lag phase data mentioned above in addition to parameters which can be obtained using pure actin filaments.

Factors that determine the value of a model. The ability of any given model to mathematically fit experimental data is only one aspect of modeling. Another aspect is the predictive power of models and the new structural insights a model gives into underlying mechanisms. The Chen et al. analysis makes no attempt to use the structural and spectroscopic data of Ca^{2+} and myosin-induced changes of the thin filament to evaluate the models. These data integrate readily with the M and G model. The M and G model has introduced several concepts that have proved very helpful in understanding the nature of the cooperative process in the thin filament: 1) the importance of K_2, discussed above, in defining the dependence of the cooperative behavior of thin filaments on the nucleotide bound to myosin; 2) the use of the apparent cooperative unit size, *n*, to define the extent to which a single myosin head can activate the thin filament (Geeves and Lehrer, 1994); and the relation of the M and G model to the Monod et al. (1965) cooperative model in which actin catalyzes the breakdown of the myosin·ADP·Pi complex, Tm is the regulatory component, and Ca^{2+} and Tn are allosteric effectors of this process (Lehrer and Geeves, 1998).

We do not believe that the M and G model is the last word on thin filament models of regulation. Indeed, we have discussed in two recent papers the limitation of any model which relies on transitions of a single A_7TmTn unit, as both the M and G and the Hill models do. We firmly believe that we need to consider Tm forming a continuous cable over the surface of actin with a finite probability of being displaced from its most favorable position into other similar energy states. The key property of Tm is the strength of the head-to-tail interactions along the cable and the flexibility/persistence length of the Tm cable (Maytum et al., 1999; Lehrer et al., 1997). This is not the place to present such ideas in detail but the work of Smith and Geeves (Smith and Geeves, 2001; Smith, 2001) shows how this might be developed.

Many other problems remain to be addressed. The problem of binding Tm to actin discussed by Tobacman and Butters (2000) is not part of the M and G model. Nor has the model been used to assess ATPase or muscle fiber regulation in any detail, as these systems remain underdefined. However, the model does provide a useful framework within which to ask mechanistic questions and to devise tests of the underlying assumptions. Despite these reservations, we believe that our three-state model will remain viable for the immediate future.

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Response to the Letter by Geeves and Lehrer

The regulation of muscle contraction is a complex process that involves changes in both the organization of the troponin subunits and the orientation of tropomyosin on actin. The changes in tropomyosin may alter the manner in which myosin binds to actin, but, in our view, the more important change is an allosteric alteration of the ability of actin to participate in the catalysis of ATP hydrolysis. Because the ATPase activity of the system is closely coupled to muscle contraction, we have used the prediction of ATPase activity as our guide to successful modeling. At the same time we recognize that it is important to be consistent with the known structural changes of the components and other data, including the manner in which myosin binds to actin. The roots of the Hill model (the model that we support), similar to that of the M and G model (McKillop and Geeves, 1993) came from an explanation of the binding of myosin to actin. The Hill model began as a description of the equilibrium binding, whereas the M and G model was fashioned around the kinetics of binding.

The following observations are our primary benchmarks: 1) inhibition of ATPase activity by tropomyosin-troponin occurs without displacement of the S1-ATP and S1-ADP-Pi complexes from actin. (2) Inhibition is characterized by a large change in the k_{cat} for ATP hydrolysis over a wide range of conditions. (3) Under conditions of high occupancy of actin sites with nucleotide-free S1, the ATPase activity is enhanced beyond that in the absence of regulatory proteins. These observations have been reviewed earlier (Chalovich, 1992). The model of Hill et al. (1980) is consistent with all of these observations (Hill et al., 1981).

The M and G model does describe the binding of myosin to actin, but it is not known if that model can predict the features of regulation of ATPase activity that were outlined

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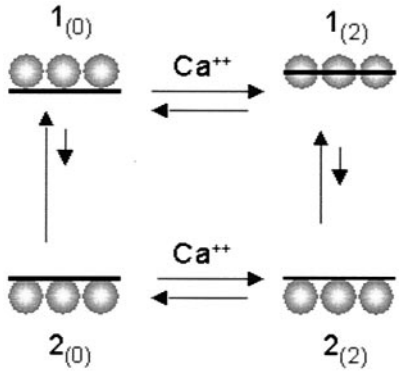

FIGURE 1 Relationship of key actin filament states of the Hill model with known structural states. Ca^{2+} and the occupancy of binding sites on actin with S1 control the state probability and the rate of ATP hydrolysis. The numbers refer to the major states of the actin filament and the subscripts define the number bound Ca^{2+}. In state $1_{(0)}$, the ATPase rate is low. S1-ATP can bind to actin in state $1_{(0)}$ whereas S1-ADP and rigor S1 bind weakly. The ATPase rate is ~ 80-fold higher with saturating Ca^{2+}, mostly state $1_{(2)}$, and the ATPase rate of state 2 (both $2_{(0)}$ and $2_{(2)}$) is approximately eightfold higher than with Ca^{2+} alone. The population of state 2 is small in the absence of S1 binding both in the presence or absence of Ca^{2+} (*arrows*). In the presence of saturating Ca^{2+}, the transition to state 2 occurs at 1 S1 per tropomyosin unit, whereas in the absence of Ca^{2+}, >2 bound S1 per tropomyosin unit are required. The ATPase rate in state 2 is at its maximum. This maximum appears to be the same regardless of the number of bound Ca^{2+} (i.e., for $2_{(0)}$, $2_{(1)}$, and $2_{(2)}$). The relative ATPase activity of states $1_{(2)}$ and $2_{(n)}$ is unknown. The blocked, closed, and open states of the M and G model correspond to our states $1_{(0)}$, $1_{(2)}$, and $2_{(n)}$. In their model, these different structural states of actin do not correspond to different potential pathways of ATP hydrolysis. Rather, an actin unit in the closed state ($1_{(2)}$) with a bound myosin molecule must progress to the open state ($2_{(2)}$) to complete ATP hydrolysis. They also assume that the properties of the open state ($2_{(n)}$) are identical to those of actin in the absence of tropomyosin-troponin. Thus, it is not possible to obtain an ATPase rate that exceeds that of pure actin in their model. The intermediate states, $1_{(1)}$ and $2_{(1)}$, are omitted from the diagram, because they are assumed to have the same properties of $1_{(2)}$ and $2_{(2)}$, respectively. To model the regulation at nonsaturating Ca^{2+} concentrations, these states may have to be included in the diagram.

above. At a minimum it seems that an allosteric change in actin activity must be incorporated into the M and G model so that the effect of Ca^{2+} on the k_{cat} for ATP hydrolysis can be simulated. The evidence for actin allostery is growing (Miki and Hozumi, 1991; Egelman, 2001). Other models incorporating allosterism, such as that proposed by Tobacman and Butters (2000), are likely to be successful in simulating the regulation of ATPase activity.

Geeves and Lehrer imply that the Hill model is inconsistent with the known structural states of the regulated actin filament. We assert that there is no inconsistency (Fig. 1). The ability of actin to accelerate the ATPase activity of myosin and the ability of muscle to contract are dependent on whether each troponin is bound to 0, 1, or 2 calcium ions. Binding of rigor type S1 to regulated actin produces an even greater ATPase activity than seen with calcium alone, and greater than that seen with pure actin. This latter point is important in that it can not be explained by simply blocking/unblocking the binding of myosin to actin by the regulatory proteins. Several examples of this potentiation of ATPase activity exist (Eisenberg and Weihing, 1970; Murray et al., 1982; Williams et al., 1988; Fredricksen and Chalovich, 2001). The structural states that have been studied thus far correspond to the low Ca^{2+}-low S1 occupancy state, the high Ca^{2+}-low S1 occupancy state, and the low Ca^{2+}-high S1 occupancy state. These three states correspond to states $1_{(0)}$, $1_{(2)}$, and $2_{(0)}$ in the Hill model where the subscripts denote the number of Ca^{2+}-ions bound to troponin. It is not known how the structure of troponin and tropomyosin is changed when only 1 Ca^{2+} is bound to troponin.

Geeves and Lehrer believe that the positions of tropomyosin are more readily explained in terms of a multiple-step binding of myosin to actin. We do not think that there are scientific grounds for making this distinction. It should be noted that incorporation of multiple-step binding into the M and G model requires some assumptions. Data supporting multiple-step binding of rigor S1 and S1-ADP to actin are strong (Trybus and Taylor, 1980; Geeves and Halsall, 1987). However, the idea that the equilibrium constant for the first process, K_1, is the same for all nucleotide states is an approximation (Taylor, 1991). Also, the M and G model incorporates a blocked state to which no myosin can bind. Yet, there are many data showing binding of S1-ATP–like states to actin in the absence of Ca^{2+}. Furthermore, in the current structural view of the regulated actin filament, none of the positions of tropomyosin overlap the putative site of electrostatic (low affinity) binding of the S1-ATP and S1-ADP-Pi states (Vibert et al., 1997). The Hill model does not assume that all myosin nucleotide complexes bind along the same two-step binding pathway and so is consistent with these and other data that show a difference between S1-ATP–like and S1-ADP–like states (Brenner et al., 1999).

It is worth reiterating that we do not take exception to two-step binding of myosin to actin. The question is: what is the relationship of this two-step binding to regulation of muscle contraction? It is not necessary to incorporate two-step binding to explain the regulation of ATPase activity (Hill et al., 1981). The Hill model was criticized because it was thought that the Hill model could not explain the kinetics of binding of myosin to actin unless multiple-step binding was included. We showed recently that the Hill model could simulate the binding kinetics with either actin or S1 in excess (Chen et al., 2001). We did additional simulations since the publication of that paper. It is also possible to simulate the data in Figure 4 in the presence of Ca^{2+} with $k_1 = 2\ \mu M^{-1}s^{-1}$ and $k_{1'} = 10\ s^{-1}$ (see Table 3 of the original paper). That is, the value of K_1 in the Hill model need not change with Ca^{2+}. *Incidentally, while responding to this letter we noticed a typographical error in Figure 1; L should be written as* β_0/α_0.

Geeves and Lehrer stated correctly that we have not modeled all of their data. It is possible that in future studies

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

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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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