Non-additivity in cation–peptide interactions. A molecular dynamics and ab initio study of Na^+ in the gramicidin channel

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The importance of non-additive energy contributions in cation–peptide interactions is investigated, in the context of Na^+ binding to the gramicidin channel, using molecular dynamics simulations and ab initio calculations. SCF supermolecule calculations are performed for an ensemble of Na^+ and N-methylacetamide complexes constructed from configurations generated by a molecular dynamics trajectory of the full channel system to represent the gramicidin binding site. A main result is that the ab initio estimates of the excess many-body interactions due to the cation are very well correlated with second-order induction energy obtained from a simple approximation based on atomic polarizabilities.

1. Introduction

Numerous fundamental biological processes involve the association of small ions such as Li^+, Na^+, K^+, Zn^{2+} or Ca^{2+} to specific protein sites, e.g., enzyme regulation, stabilization of structural elements, ion selectivity of ionophores and transport through transmembrane channels [1]. For meaningful quantitative theoretical studies of these complex systems, it is necessary to use accurate potential energy functions. Theoretical studies of biomolecular systems are generally based on pairwise additive non-bonded interatomic potential energy functions in which it is assumed that many-body effects are negligible or can be accounted for by an effective pairwise additive function [2]. While this assumption may be partly satisfied in systems involving neutral solutes and proteins [3,4], it is violated when small ions are present [5,6]. For example, because it is not possible to describe both the water molecules of the bulk phase and those in the neighborhood of an ion with a unique averaged effective potential [5,6], it is recognized that many-body effects are important for an appropriate description of ion solvation and aqueous ionic solutions [7–9]. Similarly, it may be expected that many-body effects are non-negligible when ions interact with more complex molecules such as polypeptides and proteins. Nonetheless, many-body effects in ion–peptide interactions have been generally neglected in previous studies and their importance is not known [10–12].

A first goal of this investigation is to provide a quantitative estimate of the importance of non-additive energy contributions in ion–peptide interactions. The binding of a Na^+ to the pentadecapeptide gramicidin, a well-characterized transmembrane channel [13], is chosen as an application. Non-additivity of interactions between molecules, defined as the difference between the total interaction energy and the sum over the individual pair interactions, results from quantum mechanical many-body effects and is most rigorously studied by the ab initio supermolecule approach initiated by Del Bene and Pople [14]. The approach has been used to study many-body effects in small water clusters [14–16], proton hydrates [17,18], cation–water [5,6,19,20] and anion–water systems [21]. However, in addition to the inherent difficulties caused by the very large number of atoms present in the channel system, particular problems not encountered in previous application of the supermolecule approach arise with the decomposition of non-additive energy in a

system involving a long connected polypeptide molecule. Taking advantage of the particular structure of the binding site in the gramicidin channel, the dominant contribution to the non-additive energy was estimated by performing the ab initio supermolecule calculations on complexes of Na^+ and N-methylacetamide molecules constructed by atomic substitutions from configurations generated by a molecular dynamics trajectory of the full channel system to represent the gramicidin binding site (see below).

A second goal of this investigation is to examine the validity of simple approximations to account for the dominant many-body effects in the presence of an ion, in particular those retaining the pairwise additive form of the peptide–peptide and water–peptide potential function. The motivations for such an approach are obvious. First, addition of many-body effects to biomolecular simulations represents a significant increase in computational cost [4,8]. Second and perhaps even more important, the efforts needed to develop and parametrize a potential function for biomolecular systems accounting for many-body effects should be expected to be no less than the major and untrivial efforts that were required for developing and parametrizing the effective pairwise additive force fields used currently [22–26]. In the present investigation, a simple potential function representing the dominant "excess" many-body effects due to the ion in terms of second-order induced polarization energy is examined. Such approximation is supported by the findings of previous ab initio supermolecule studies of water–water and cation–water systems, which indicated that the major non-additive contribution is generally caused directly by the ion [5,6]. A good agreement is found between the ab initio and the potential function.

The procedure used to study the non-additive energy contribution due to the ion consists of two steps. First, a molecular dynamics trajectory of Na^+ in the fully solvated gramicidin channel is performed to generate an ensemble of instantaneous configurations of the ion and its ligands in the binding site. Second, ab initio supermolecule calculations are performed on a selected subset of atoms including only the nearest ligands to estimate the dominant non-additive contribution for the ensemble of configurations. This procedure differs with previous applications of the supermolecule approach in which the potential surface was explored systematically [5], or optimized geometries were examined [21]. Due to the large number of degrees of freedom involved in the channel system, it is not possible to perform a systematic exploration of the potential surface. An approach based on a thermal ensemble generated from a molecular dynamics trajectory is more advantageous for such a large system. It provides an effective way of sampling the most relevant regions of the multidimensional configurational space. Furthermore, it allows the evaluation of the fluctuations of the ab initio non-additive energy, which can be added as a correction to the free energy.

Details about the computation are given in section 2. The classical electrostatic treatment of induced polarization, the microscopic solvated channel system and the supermolecule ab initio calculations of the Na^+ and four NMA complexes are described. The non-additive energy is calculated at the Hartree–Fock SCF level for 36 complexes using the 3-21G basis set and four complexes using the 6-31G* basis. Basis set superposition errors are corrected with the counterpoise method [27]; overestimated bond polarities are corrected using an empirical scaling procedure based on the experimental dipole moment of NMA [28]. The corrected SCF supermolecule calculations are found to agree well with second-order induced polarization calculated from the potential energy function. The chemical potential of Na^+ in the binding site of the gramicidin channel is estimated by a slow growth procedure and free energy perturbation theory. A brief conclusion ends the Letter.

2. Computational details

2.1. Excess ion-induced polarization energy

It is assumed that the "excess" non-additive many-body effects associated with the presence of the ion can be expressed in terms of ion-induced polarization on the peptide atoms, i.e. only the ion is allowed to induce a dipole on polarizable peptide atoms. From classical electrostatics, the dipole induced by the charge q_{ion} of the ion on a peptide atom i is

$$\boldsymbol{\mu}_i = \alpha_i q_{\mathrm{ion}} \frac{(\boldsymbol{r}_i - \boldsymbol{r}_{\mathrm{ion}})}{|\boldsymbol{r}_i - \boldsymbol{r}_{\mathrm{ion}}|^3}, \qquad (1)$$

where α_i is the atomic polarizability of atom i. The induction energy is approximated, to first order, by the "ion–induced-dipoles" interaction,

$$E_{\rm pol}^{(1)} = \sum_i -\frac{1}{2}\frac{q_{\rm ion}^2 \alpha_i}{|r_i - r_{\rm ion}|^4}, \quad (2)$$

to second order, by "induced-dipoles–partial charges" interactions,

$$E_{\rm pol}^{(2)} = \sum_{ij} -q_j \frac{r_{ij}\cdot\mu_i}{|r_{ij}|^3}, \quad (3)$$

and to third order, by "induced-dipoles–induced-dipoles" interactions,

$$E_{\rm pol}^{(3)} = \sum_{ij} \frac{\mu_i\cdot\mu_j}{|r_{ij}|^3} - 3\frac{(\mu_i\cdot r_{ij})(r_{ij}\cdot\mu_j)}{|r_{ij}|^5}, \quad (4)$$

where the q_j represents all partial charges in the system and $r_{ij}=(r_i-r_j)$.

The first-order polarization, $E_{\rm pol}^{(1)}$ results in a pairwise contribution to the energy of the system; the second- and third-order terms $E_{\rm pol}^{(2)}$ and $E_{\rm pol}^{(3)}$ correspond to non-pairwise additive corrections to the potential energy function. The first- and second-order polarizations were included in previous studies of the gramicidin channel [12,29]. In the present investigation, potential functions including the first-, second- and third-order polarization are called $E_{\rm pol}^{(1)}$, $E_{\rm pol}^{(12)}$ and $E_{\rm pol}^{(123)}$.

Atomic polarizabilities were assigned to all non-hydrogen peptide atoms to reproduce the ab initio potential energy surface involving one N-methylacetamide molecule and Na^+ [28]. Their values are similar to other atomic polarizabilities: α is 0.3 for C, 0.5 for methyl groups, 1.5 for N and 1.5 for O [22]. All intramolecular polarization interactions involving pairs of atoms directly bonded to one another, or pairs of atoms separated by two or three bonds are excluded (1–2, 1–x–3 and 1–x–x–4 bonded pairs); no polarizability is assigned to the TIP3P water atoms. All other parameters have been given elsewhere [30].

2.2. Gramicidin channel system and simulation procedure

More details about the simulations will be given elsewhere [29]. Briefly, the gramicidin channel system contains one Na^+, 189 TIP3P water molecules and 88 hydrophobic carbon spheres included to provide a model membrane. All non-polar hydrogens not involved in hydrogen bonding were included into the heavy atom to which they are attached, in an extended atom representation. The channel was constructed as a right-handed $\beta_{6.3}$ head-to-head dimer [31,32]. In previous calculations, the binding site of Na^+ was found at 9.2 Å from the center of the dimer [12], in good agreement with experimental observations [33–35].

Dissipative and stochastic Langevin forces corresponding to a heat bath temperature of 300 K and a velocity relaxation rate of 40 ps^{-1} were applied to the hydrocarbon-like LJ spheres, the water molecules in the bulk-like regions, and the side chains of the channel. To maintain the orientation of the channel in the finite system, a cylindrical harmonic restraining potential was applied to the center of mass of the gramicidin monomers. The non-bonded electrostatic interactions were calculated without truncation using an "extended electrostatics method" [36]. The integration time step was 0.001 ps for the dynamics.

Two trajectories of 15 ps were calculated to increase the extent of the configurational sampling for the ab initio supermolecule calculations. Trajectory 1 included only first-order polarization in a pairwise additive potential approximation (model $E_{\rm pol}^{(1)}$), trajectory 2 included first- and second-order polarization resulting in a non-additive potential (model $E_{\rm pol}^{(12)}$); 11 configurations were extracted from trajectory 1 and 25 configurations were extracted from trajectory 2 for the ab initio supermolecule calculations. Because it is generated model $E_{\rm pol}^{(12)}$, the configurations extracted from trajectory 2 provide the most representative ensemble of ion–ligand complexes at room temperature. However, configurations corresponding to large values of the non-additive energy occur infrequently during the course of this trajectory. To better explore the correlation between the supermolecule calculations and the approximate potential function, it is of interest to enhance the sampling of ion–ligand configurations corresponding to large non-additive energy. Such configurations are more probable during the course of trajectory 1 because it is generated with $E_{\rm pol}^{(1)}$. The ensemble of 36 configurations allows an effective exploration of non-additive effects with the supermo-

lecule calculations. Furthermore, the configurations from both trajectories are used in eq. (5) to estimate the contribution of $E_{pol}^{(2)}$ to the free energy (see below).

The free energy associated with the process of charging the Na^+ ion in the binding site was calculated with the slow growth method [37]. Forward and backward trajectories of 50 ps with the potential function $E_{pol}^{(1)}$ were used. The first trajectory (the charge goes from 0 to1) yielded -142 kcal/mol and the second trajectory (the charge goes from 1 to 0) yielded -136 kcal/mol. The contribution of $E_{pol}^{(2)}$ to the total free energy was estimated from perturbation theory

$$\mathscr{A}_{pol}^{(2)} = \tfrac{1}{2}[-k_B T \ln\langle \exp(-E_{pol}^{(2)}/k_B T)\rangle_{(1)} + k_B T \ln\langle \exp(+E_{pol}^{(2)}/k_B T)\rangle_{(2)}], \quad (5)$$

from averages obtained with trajectory 1 and trajectory 2. The calculated value of $\mathscr{A}_{pol}^{(2)}$ is $+45$ kcal/mol. Finally, a continuum electrostatic correction of -9 kcal/mol was added to compensate for the finite size of the simulation system (see ref. [29] for details). The resulting total free energy of Na^+ in the binding site of the gramicidin channel is -103 kcal/mol.

2.3 Ab initio calculations

To perform the ab initio calculations, it is necessary to select a sub-system with a reasonable number of atoms. The particular ligand structure around the cation in gramicidin is advantageous: in the binding site the ion makes close contact with four carbonyls oxygen, D-Val_8, D-Leu_{10}, L-Trp_{13} and L-Trp_{15}, and two water molecules [12]. The four amino acids are not directly chemically bonded to one another. The dominant contribution to the non-additive energy was calculated by substituting the four nearest backbone carbonyls by N-methylacetamide molecules (NMA) (see fig. 1). The C^β of the side-chains were replaced with hydrogens. For example, the first NMA molecule was substituted for the atoms of D-Val_8 and L-Trp_9, i.e. C_8^α was substituted by CH_3, C_8 by C, O_8 by O, N_9 by N, H_9 by H and C_9^α by CH_3. All other atoms of the system, including the water molecules, were removed. Hydrogens of the two methyl groups were constructed with bond length of 1.107 Å and bond angles of 108.53°; the dihedrals were chosen to represent a peptide backbone in a fully extended conformation with dihedral angles ϕ and ψ equal to 180°. There are 21 heavy atoms in each complex used in the supermolecule calculations. One typical example of a complex of Na^+ with four NMA is shown in fig. 1; the coordinates were obtained from trajectory 2 and correspond to configuration 3 of table 1. Similar atomic substitution methods have been used in combined quantum mechanical and molecular mechanical simulations (QM/MM) [39], or the

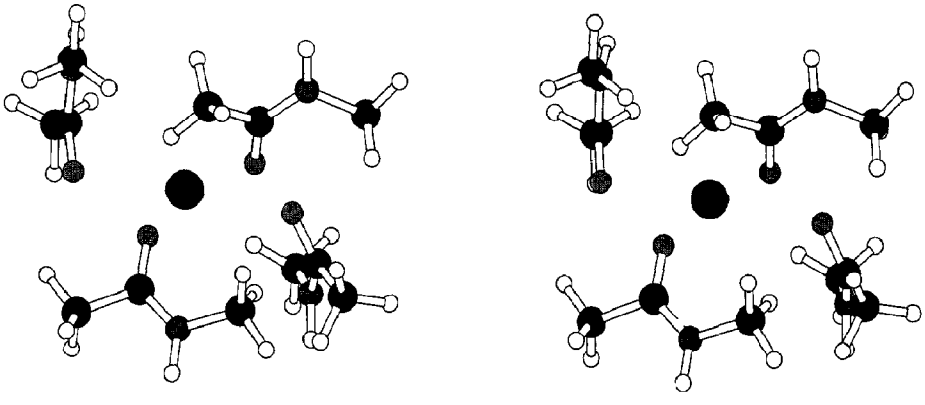

Fig. 1. Stereo representation of the complex of Na^+ and 4 NMA molecules made to represent the ion binding site in the gramicidin channel. The four carbonyls oxygen are from D-Val_8 (bottom left), D-Leu_{10} (bottom right), L-Trp_{13} (top left) and L-Trp_{15} (top right). The axis of the channel is oriented vertically, the N-terminus located towards the top of the picture. The coordinates correspond to the configuration 3 of table 1 obtained from trajectory 2 using second-order induction $E_{pol}^{(12)}$. The picture was made with the program CHIRON [38].

Table 1
Energy decomposition of four configurations (kcal/mol) [a]

No.	Models	$\Delta\Delta E_{1234}$	ΔE_{1234}	$\Sigma_i \Delta E_i$	ΔE_1	ΔE_2	ΔE_3	ΔE_4
1	HF/6-31G*	33.57	−111.03	−144.60	−37.89	−33.79	−36.21	−36.70
	HF/3-21G	29.68	−128.07	−157.75	−41.16	−36.25	−39.68	−40.66
	$E_{pol}^{(12)}$	33.61	−105.08	−138.69	−35.58	−32.45	−35.07	−35.58
	$E_{pol}^{(123)}$	46.21	−92.48	−138.69	−35.58	−32.45	−35.07	−35.58
2	HF/6-31G*	31.06	−114.19	−145.25	−37.93	−35.42	−33.93	−37.97
	HF/3-21G	26.75	−130.87	−157.61	−41.67	−38.09	−36.75	−41.10
	$E_{pol}^{(12)}$	30.15	−108.24	−138.39	−36.10	−33.75	−33.27	−35.27
	$E_{pol}^{(123)}$	41.15	−97.24	−138.39	−36.10	−33.75	−33.27	−35.27
3	HF/6-31G*	23.33	−96.88	−120.20	−35.47	−25.44	−24.03	−35.26
	HF/3-21G	19.49	−109.17	−128.66	−38.65	−26.60	−25.02	−38.38
	$E_{pol}^{(12)}$	20.43	−94.34	−114.77	−34.38	−23.32	−23.26	−33.81
	$E_{pol}^{(123)}$	27.90	−86.86	−114.77	−34.38	−23.32	−23.26	−33.81
4	HF/6-31G*	15.68	−87.26	−102.94	−30.72	−33.10	−4.52	−34.59
	HF/3-21G	12.84	−97.13	−109.97	−32.70	−34.99	−4.92	−37.35
	$E_{pol}^{(12)}$	12.34	−83.98	−96.32	−29.13	−30.03	−4.39	−32.76
	$E_{pol}^{(123)}$	16.97	−79.35	−96.32	−29.13	−30.03	−4.39	−32.76

[a] All ab initio calculations were corrected for BSSE and overestimated bond polarities.

sum of interactions between fragments computed ab initio procedure (SIBFA) [40].

In the substituted $(NMA)_4$ system the total ion–ligands interaction energy is

$$\Delta E_{1234} = E[\text{ion}, \text{NMA}_1, \text{NMA}_2, \text{NMA}_3, \text{NMA}_4] - E[\text{NMA}_1, \text{NMA}_2, \text{NMA}_3, \text{NMA}_4] - E[\text{ion}] . \quad (6)$$

To extract the "excess" non-additive energy due to the presence of the cation, ΔE_{1234} can be decomposed in terms of the four individual interaction energies, ΔE_i,

$$\Delta E_i = E[\text{ion}, \text{NMA}_i] - E[\text{ion}] - E[\text{NMA}_i] , \quad (7)$$

and a remaining part, $\Delta\Delta E_{1234}$, representing the non-additive contribution to the total interaction energy, that is,

$$\Delta\Delta E_{1234} = \Delta E_{1234} - \sum_{i=1}^{4} \Delta E_i . \quad (8)$$

In contrast with previous studies [5,6,14,16,20], a complete decomposition of the internal NMA–NMA non-additive interactions was not done. Only the excess non-additivity associated with the presence of the ion was extracted. To estimate the excess non-additive contribution, it was necessary to perform 10 ab initio single-point energy calculations for each configuration of the ion–$(NMA)_4$ system. The non-additive energy contribution was calculated using eq. (8) with the ab initio energies at the Hartree–Fock SCF level using the 3-21G basis set, and with the empirical energy function. Previous studies have shown that the Hartree–Fock level can reproduce correctly the main part of the non-additive energy and that correlation energy is of minor importance in ion–water systems [41,42]. It was found, from preliminary calculations on the 36 configurations with the 3-21G basis set, that the non-additive energy varies approximately from 15 to 34 kcal/mol. To obtain more accurate estimates, four configurations of the ion–$(NMA)_4$ complex spanning the observed range of the non-additive energy were chosen to perform 6-31G* calculations. The ion–ligand complex of configuration 3 is shown in fig. 1. All calculated values were corrected for basis set superposition error (BSSE) by the counterpoise method [27]. To account for overestimated bond polarities, the resulting energies were scaled by the ratio of the experimental to the ab initio dipole of the NMA molecule [28]; the scaling factors are 0.97 and 0.91 for the 3-21G and 6-31G* basis sets. Generally, the BSSE and

scaling corrections decreased non-additive energy, $\Delta\Delta E_{1234}$ (by 8 to 10 kcal/mol for 3-21G and 5 to 6 kcal/mol for 6-31G*), and the pair interactions, ΔE_i (by 8 to 10 kcal/mol for 3-21G and 5 to 6 kcal/mol for 6-31G*). For example, taking the 6-31G* calculations of configuration 1 in table 1, the corrections changed $\Delta\Delta E_{1234}$ from 39.25 to 33.57 kcal/mol and ΔE_1 from -42.15 to -37.89 kcal/mol.

Calculations were done on a SGI/480 and on the C90 at the PSC using the program GAUSSIAN 90 [43]. The calculations necessary for one configuration took approximately 4 h on a SGI/480 for the 3-21G; and 45 min on the C90 for the 6-31G*.

3. Results and discussion

The detailed energy decomposition with four configurations is given in table 1. Although the configurations extracted from trajectory 2 are more representative of the ion–ligand complex at room temperature, the configurations extracted from trajectory 1 were also considered for supermolecule calculations to better explore the importance of non-additive effects. Configurations 1 and 2, generated with $E_{\rm pol}^{(1)}$, yield stronger cation–NMA interactions and a larger non-additive energy than configurations 3 and 4, which were generated with $E_{\rm pol}^{(12)}$. On average, the ab initio 6-31G* non-additive energy constitutes 21% of the total interaction energy for the ion–$(NMA)_4$ complexes for the two configurations extracted from trajectory 2. This value may be compared with the value of 10% to 15% per water molecule reported for cation–water complexes [6].

The ab initio calculations at the SCF level with the 3-21G and 6-31G* basis do not provide quantitative estimates of the pair interactions and the non-additive energies. As observed in previous studies [28], the ion–NMA pair interaction energies are generally overestimated and significant counterpoise corrections are necessary to provide meaningful results. Furthermore, although the 3-21G and 6-31G* calculations are well-correlated, it is found that the 3-21G basis systematically underestimates the non-additive energy and overestimates the interaction energy relative to the 6-31G* basis. Extensive convergence studies on basis set dependence and electron correlation with Møller–Plesset perturbation would be necessary to provide more definitive estimates. Nevertheless, given the other approximations involved in the present study, the ab initio calculations corrected for systematic biases provide a semiquantitative estimate of the importance of non-additive energy in ion–peptide interactions.

The comparison of the ab initio calculations with the potential energy function including second-order induction energy is shown in figs. 2 and 3. To provide a more extensive set of ab initio estimates for the figures, the 3-21G calculations were empirically scaled to fit the 6-31G* results; scaling factors of 1.16 and 0.92 were used for $\Delta\Delta E_{1234}$ and ΔE_i, respectively. It is observed in fig. 2 that the excess non-additive component is very well correlated with the second-order induction energy estimated from classical electrostatics and atomic polarizabilities. The best agreement with the ab initio calculations is obtained with the second-order polarization; addition of a third-order correction ("induced-dipole–induced-dipole" interaction) tends to overestimate the

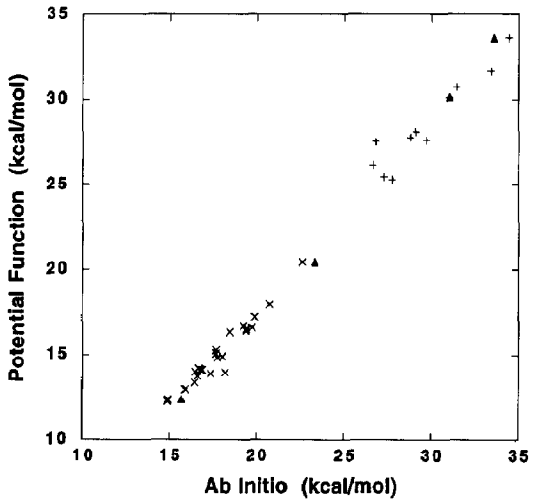

Fig. 2. Excess ion-induced non-additive energy, $\Delta\Delta E_{1234}$. Scatter plot of the ab initio results corrected for systematic biases and empirical potential function interaction energy, $E_{\rm pol}^{(12)}$. The HF/3-21G energies were scaled by a factor of 1.16 (obtained by least-squares fit) to account for the underestimate in non-additive energy relative to the 6-31G* basis set. A least-squares fit yields a slope of 0.91. The 25 and 11 configurations extracted from trajectory 1 and 2 for the HF/3-21G calculations are indicated by (+) and (×), respectively; the four HF/6-31G* calculations are indicated by triangles.

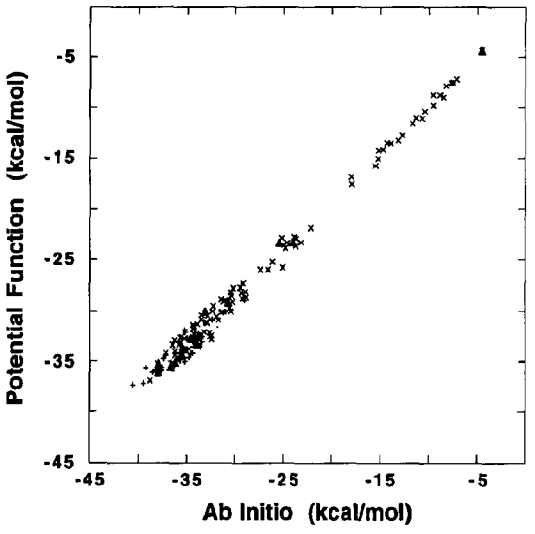

Fig. 3. Individual Na^+–NMA interactions, ΔE_i. Scatter plot of the ab initio results corrected for systematic biases and empirical potential function interaction energy, $E_{pol}^{(12)}$. The energies of the 3-21G basis set were scaled by a factor of 0.92 (obtained by least-squares fit) to account for the overestimate in interaction energy relative to the 6-31G* basis set. A least-squares fit yields a slope of 0.95. The 25 and 11 configurations extracted from trajectory 1 and 2 for the HF/3-21G calculations are indicated by (+) and (×), respectively; the four HF/6-31G* calculations are indicated by triangles.

non-additive energy. A least-squares fit of $\Delta\Delta E_{pol}^{(123)}$ yields a slope of 1.24, while $\Delta E_{pol}^{(12)}$ yields a slope of 0.91. The better agreement with the lower order is fortunate but may have been caused by a cancellation of errors. The parametrization of the ion–peptide potential function was based on ab initio calculations of Na^+ with NMA, and there were no attempts to adjust the atomic polarizabilities assigned to the peptide atoms to best fit the excess non-additive contribution. It is remarkable that satisfactory agreement is obtained even without any extensive effort to refine the parameters. In fact, adjusting the values of α_i to 0.5 for C, 0.6 for methyl groups, 1.5 for N and 1.7 for O yields a much improved agreement, with a slope of 0.95 for $\Delta\Delta E$ and a slope of 1.0 for ΔE. Thus, the non-additive contribution and the interaction energy may be both improved by very small adjustments of the parameters.

Because all non-bonded interactions involving 1–2, 1–*x*–3 and 1–*x*–*x*–4 bonded pairs were not included in the electrostatic polarization energy, the Na^+–NMA potential function is equivalent to simple pairwise additive approximation, i.e. there are no "induced-dipole–partial charge" interactions in the isolated Na^+–NMA complex. Although there are no fundamental justifications for excluding those pairs, the good agreement observed in fig. 3 between the potential function and the ab initio estimates for Na^+–NMA interactions indicates that the individual cation–NMA interactions are generally well reproduced by this approximation. However, use of a pair additive approximation leads to a gross overestimation of the interaction energy in the ion–$(NMA)_4$ complex and in the gramicidin channel system. The calculated free energy of Na^+ in the binding site is −148 kcal/mol when only first-order polarization energy is included, i.e. nearly 45% too large. A value of −103 kcal/mol, in good agreement with experimental estimates [44], is obtained after including the second-order polarization $E_{pol}^{(2)}$. The large contribution to the free energy is partly due to the effects of fluctuations. For example, using trajectory 2 generated with $E_{pol}^{(12)}$, the total non-additive energy for the full channel system is −24.5 kcal/mol on average with rms fluctuations of 4.5 kcal/mol. To second order in a cumulant expansion, this represents approximately a free energy difference of $-[-24.5-(4.5)^2/2k_BT] = +41.5$ kcal/mol, close to the calculated value of 45 kcal/mol. This illustrates the importance of considering the fluctuations of non-additive contributions. A second contribution to the free energy arises from the two water molecules near the ion (see table 2). Even though no atomic polarizabilities were assigned to the water atoms, there is an unfavorable non-additive interaction between the water partial charges and the dipoles induced by the ion on the peptide atoms; from the potential function this contribution is +12 kcal/

Table 2
Average ion–ligand distances in the binding site (Å)

Ligands	$E_{pol}^{(1)}$ (traj. 1)	$E_{pol}^{(12)}$ (traj. 2)
H_2O (in)	2.24±0.08	2.26±0.08
H_2O (out)	2.27±0.09	2.27±0.09
C=O D-Val_8	2.28±0.11	2.54±0.29
C=O D-Leu_{10}	2.28±0.12	2.41±0.15
C=O L-Trp_{13}	2.32±0.14	3.49±0.43
C=O L-Trp_{15}	2.27±0.11	2.41±0.18

mol on average in the channel system during trajectory 2. In future work the influence of the nearest waters in the ab initio supermolecule energy decomposition will be considered explicitly.

To investigate the significance of the approximations involved in effective pairwise potentials, a numerical experiment was attempted in which the atomic polarizabilities, α_i, in the pairwise additive model $E_{\rm pol}^{(1)}$ were adjusted to reproduce the correct charging free energy of Na^+ in the binding site. Using trajectory 2, generated with the non-pairwise additive model $E_{pol}^{(12)}$, the free energy difference,

$$\Delta\mathscr{A}_{12-1}(\alpha_i) = -k_{\rm B}T\ln\langle\exp[-(E_{\rm pol}^{(1)}(\alpha_i) - E_{\rm pol}^{(12)})/k_{\rm B}T]\rangle_{(2)}, \quad (9)$$

was minimized by decreasing the α_i by a factor of approximately 2 in $E_{\rm pol}^{(1)}$. The result is an effective pairwise potential with a set of atomic polarizabilities, α_i, reproducing the correct charging free energy of Na^+ in the binding site. However, further analysis shows that the approximation is inappropriate. The individual Na^+–NMA interactions, estimated with configurations extracted from trajectory 2, are underestimated by 15% to 20% on average. Moreover, the optimized geometry of an isolated Na^+–NMA complex is incorrect: the ion–oxygen distance is 2.20 Å and the interaction −31.2 kcal/mol with the effective pairwise additive potential function, while an optimized ion–oxygen of 2.10 Å and an interaction of −37.8 kcal/mol should be found [28]. This numerical experiment shows that an effective pairwise potential function can be parametrized to reproduce global properties such as the charging free energy, but that this is done at the expense of an accurate description of the individual interactions. For a valid model of ion–peptide interactions, it is necessary to account for non-additive polarization effects.

The many-body forces have also observable structural consequences. There is a small shift of the average position of the ion along the axis of the channel, from 9.2 Å (trajectory 1) to 9.4 Å (trajectory 2). Although the main ion–carbonyl contacts are maintained, the average ion–ligand distances, given in table 2, are increased when the non-additive energy contribution is included. Similar structural changes have been observed in ab initio calculations of ions in small water clusters [6] and in molecular dynamics simulations of ions in bulk water [7,8]. The largest perturbation involves L-Trp_{13}. In trajectory 1, it is the weakest ion–ligand pair in the binding site, exhibiting the largest distance (2.32 Å) and fluctuations (0.14 Å rms). In trajectory 2, the average distance is 3.49 Å and the contact is essentially lost. Generally, the increased ion–ligand distance caused by the non-additive energy can be understood as a result of the effective repulsion between an electronegative ligand close to the cation and the dipoles induced by the cation on the other neighboring ligands (pointing away from the cation). The case of L-Trp_{13} provides an extreme example of the importance of many-body forces on the ion–ligand structure. In a flexible peptide system, the detailed ion–ligand structure may be very sensitive to many-body effects.

4. Conclusion

The importance of the excess non-additive energy contribution in ion–peptide interaction was investigated using molecular dynamics simulations and ab initio calculations. Supermolecule calculations were performed at the SCF level for an ensemble of ion–ligand complexes, constructed by atomic substitutions from configurations generated by a molecular dynamics trajectory of the full molecular system. In this particular application four backbone carbonyl ligands were substituted by NMA molecules to study Na^+ binding in the gramicidin channel, but the approach could be used in other peptide systems. This differs from previous applications of the SCF supermolecule approach which were mostly based on a systematic exploration of the potential surface [5], or on geometry optimized configurations [21]. For investigating a flexible peptide system with a large number of degrees of freedom, the thermal ensemble is more advantageous.

The calculated ab initio values, corrected for systematic biases, are found to be in qualitative agreement with the results given by a potential function based on second-order induced polarization energy. It is a main result of this Letter that such a simple treatment based on classical induced dipoles with atomic polarizabilities is able to account semiquan-

titatively for the excess non-additive energy contribution in ion–peptide interactions. Including this correction to the potential function leads to a dramatic change of the charging free energy of Na^+ in the gramicidin binding site, i.e. from -148 to -103 kcal/mol. Non-additivity is essential for a valid description of ion–peptide interactions. The parameters of an effective pairwise potential function, adjusted to reproduce the free energy of charging in the binding site, resulted in a poor description of the individual Na^+–NMA interactions.

Many-body effects are overestimated if third-order polarization contributions are included. This may be caused by a cancellation of errors. In a more rigorous description, the induced dipoles should be determined self-consistently. However, this is difficult to achieve without fundamental changes in the parametrization of the peptide potential function. The approach described here represents a convenient approximation that is computationally simple. Comparison with ab initio estimates shows that the approximation captures the essential aspects of the excess non-additive energy contributions. In future work, the investigation of many-body effects in biological systems will be extended to other monovalent and divalent cations and to include the influence of water.

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