Effects of electric charges on hydrophobic forces

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Effects of electric charges on solvent-induced forces (SIFs) among hydrophobic solutes in explicit water are studied by molecular dynamics simulations. It is shown that (i) a unitary charge on one element of a pair of Lennard-Jones (LJ) solutes causes strong, sign-dependent, repulsive contributions to SIFs; (ii) SIFs between two LJ particles undergo strong, nonadditive, sign-dependent modulation in the presence of a third electrically charged LJ solute. The physical origin of the observed effects and their key biological relevance is discussed. [S1063-651X(97)50111-5]

Solvent-induced interactions and their most familiar subset, hydrophobic interactions [1], are quantitatively expressed in terms of solvent-induced forces (SIFs) and related potential of mean force (PMF) [2]. As a consequence of the known many-body character of PMF, when a number of solutes (or parts of composite solutes such as proteins) is present, pair additivity of SIFs should not be expected. In consideration of the relevant role of solvent in protein folding [3] and function [4], investigations of the size and quality of effects due to such many-body character of PMF and SIFs cover a great potential interest. Time- and space-resolved views of SIFs can be obtained from molecular dynamics (MD) simulations taking explicitly into account the molecular nature of the solvent [5]. Although costly in computer time, this approach is free of approximations and offers uniquely detailed complements to experimental studies, at the microscopic spatial resolution relevant to the well known high specificity of biofunctional interactions [6–9]. Recent work of this type has elicited the exceptionally strong nonadditivity [6], context dependence and long-range correlated or relayed action [7,8] of microscopically space resolved SIFs in aqueous solutions. These properties endow SIFs acting on local sites of complex solutes with exquisite specificity, potentially highly relevant to biofunction. In the present MD study we elicit in simple solute configurations the change of hydrophobic forces caused by electric charges and its dependence upon charge sign. Specifically, we show that (i) a charge on one element of a pair of Lennard-Jones (LJ) solutes causes large repulsive contributions to SIFs within the pair and (ii) SIFs between a pair of apolar LJ solutes are markedly changed in a nonadditive and distance-dependent way by the presence of a third LJ solute bearing an electric charge. Both effects depend strongly on charge sign. This agrees with the known differences in hydration of positively and negatively charged solutes [10,11] and with recently reported different interactions of positively and negatively charged protein residue sidechains with water [7]. Sign dependence, not expected in continuum models, is caused by the asymmetric distribution of charges on the water molecule.

SIFs acting on a solute are the sum of microscopic forces exerted on it by all solvent molecules, thermodynamically averaged over all solvent configurations [2]. They are caused by perturbations (due to solutes) of the potential energy surface in the multidimensional configurational space, and consequent perturbation of statistically populated solvent configurations [6,7]. Such perturbations are the combined result of excluded volume and solute-solvent interaction potential and are the origin of hydration and related free energy [6,8]. SIFs can be viewed as the result of hydration overlaps [12]. The negative gradient of the hydration free energy (or, equivalently, of the solvent induced contribution to PMF) with respect to the coordinates of a given solute expresses the SIF acting on it [2].

Our simulation box (a cube with 21.75 Å side) contained 343 molecules (water+solutes) at an average temperature of 298 K. Periodic boundaries, microcanonical (NVE) ensemble, and Ewald sum techniques were used. For water-water interactions we used the TIP4P potential [13], particularly appropriate in the case of ion-water solutions [13,14]. Solutes, held in fixed positions, were modeled as LJ spheres having the same potential parameters of oxygen in the TIP4P model ($\epsilon = 0.64857$ kJ mol^{-1} and $\sigma = 3.154$ Å). When appropriate, a unitary electronic charge of either sign was attributed to one of the LJ solutes, and neutralized by an evenly distributed charge density of opposite sign [15]. Independent runs of not less than 800 and up to 1200 ps were obtained for each configuration of solutes. For better statistical significance, each run was obtained as a sequence of 200 ps trajectories decoupled from each other by 20 ps annealings at 800 K, followed by 40 ps equilibration at 298 K. SIFs on each solute can be computed as time averages of instantaneous force vectors exerted upon that element by all water molecules [5–7]. Direct solute-solute forces were not taken into account. Related errors ($\leqslant 4$ pN) and statistical significance were evaluated as in [16] and as in [6], with identical results. The total force and torque acting on all solutes were seen to be zero within the same accuracy. In order to obtain hydration patterns, the simulation box was divided into cells of 0.7 Å side length and the probability P (normalized to bulk water) that the center of an oxygen atom of a solvent molecule fell within the cell was computed [6]. Using this space-occupancy probability and the interaction potential, contributions from each cell to SIFs acting on LJ solutes could be computed and summed up as an equivalent way to obtain SIFs. For SIFs acting on charged solutes,
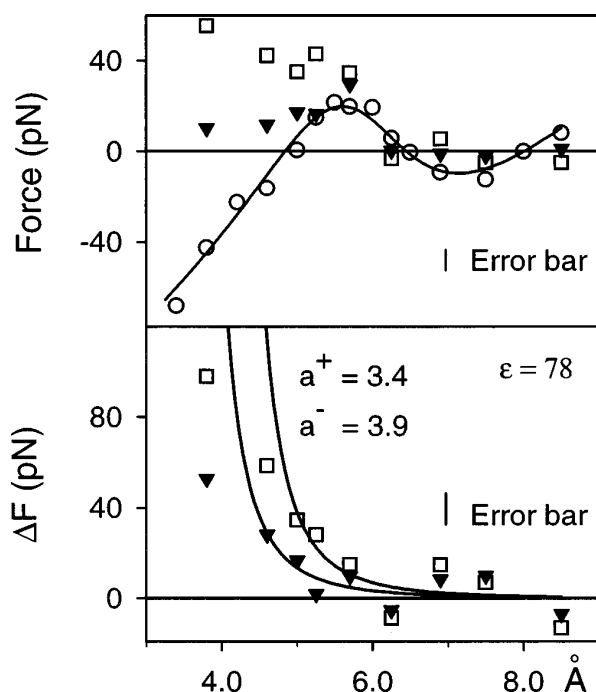
FIG. 1. Solvent-induced forces (SIFs) vs center-to-center distance between two Lennard-Jones solutes in a bath of TIP4P water. Top: Circles refer to electrically uncharged solutes (the continuous line is a guide to the eye); triangles refer to the case of a unitary positive electronic charge added to one of the solutes; squares, as above, for a negative charge. Bottom: Triangles and squares: as above, after subtraction of hydrophobic SIFs for uncharged solutes. Continuous lines: force between a unitary charge and a sphere calculated by classic electrostatic, in a continuum polarizable medium with $\epsilon = 78$. Different sphere radii, $a^+ = 3.4$ and $a^- = 3.9$ Å, are obtained by best fittings of data relative to a positively or negatively charged solute, respectively.

both probabilities of the space occupancy by oxygen and hydrogen atoms of the solvent were used.

In a first set of runs, the water bath contained a pair of uncharged LJ solutes in fixed positions, at several solute-solute distances. Force values shown in Fig. 1, top (circles) exhibit the known oscillatory distance dependence of hydrophobic forces, reflecting the particulate nature of the solvent [17]. In a second set of runs, one of the two solutes was bearing, in turn, a unitary positive or negative charge. A repulsive SIF was now observed, rapidly decreasing at increasing distance, so as to fade out at $d > 6$ Å. As evidenced in Fig. 1, bottom, the additional repulsive contribution overwhelms at short distances the attractive hydrophobic force. Its size (but not its sign) depends on charge sign, the effect of the negative charge being consistently larger. In Fig. 1, bottom, these charge contributions are compared with the force, calculated in terms of classical electrostatics, between a point charge and a spherical cavity, in a continuum polarizable medium, with $\epsilon = 78$ and optimal sphere radii determined by best fitting. At sufficiently large distance ($d > \approx 4.5$ Å), MD data follow a trend similar to that of the continuum model. However, an unphysical choice of different cavity radii in the cases of positive and negative charge is required by fitting. Best fitting radii are 3.4 and 3.9 Å, respectively, to be compared with the location of the first $g_{\text{LJ-0}}(r)$ maximum [11] around the uncharged LJ solute ($R = 3.38$ Å). Alterna-

tively, different effective dielectric constants or charge values are obtained from best fits if one given radius is used for both solutes. Even when using such *ad hoc* fitting procedures, strong deviations from the continuum model description are in any case observed at short distances (Fig. 1, bottom).

Another set of simulations concerns three solutes in configurations of Fig. 2. Solutes 1 and 2 are uncharged, at a fixed 5 Å distance corresponding to a zero pairwise SIF between them, as per data in Fig. 1. Solute 3 was fixed on the axis of the 1-2 segment and could bear in turn a null, positive, or negative charge (left to right). In the figure we show the cases of $d_{1\text{-}3} = d_{2\text{-}3} = 4.6$ Å (top) and $d_{1\text{-}3} = d_{2\text{-}3} = 5.7$ Å (bottom). Vectorial SIFs are indicated on each solute as obtained from simulations (gray), and as additively computed (black) from pairwise data of Fig. 1. We call F_x (1-2) the SIF component on solutes 1 and 2 along the 1-2 direction. Solute 3, even if uncharged, causes a sizeable F_x (1-2). This force, whose sign and value are distance dependent, is enhanced by nonadditive contributions up to threefold (30 versus 9 pN at 5.7 Å) in agreement with results in Refs. [4] and [6]. Addition of a charge on solute 3 causes still another, strongly sign dependent, change of F_x (1-2). At 4.6 Å, we obtain ≈ 0 and -25 pN for positive and negative charge, respectively (versus $+21$ pN for uncharged solute 3). The force acting on solute 3, F_y (3) (orthogonal to the 1-2 direction) expresses the SIF caused by the hydrophobic 1-2 pair on solute 3. Similar strong nonadditive and charge sign dependent effects are seen on F_y (3). At 4.6 Å its values are -15 and -70 pN for positive and negative charge, respectively (versus $+35$ pN for uncharged solute 3). Nonadditive contributions (highest at 5.7 Å) can go up to a factor 2.

The origin of these effects is made clear in Fig. 3. Here we see (top) how the hydration pattern (visualized by a space occupancy isoprobability surface, with P defined as above) is affected by unitary charges of either sign on solute 3. The
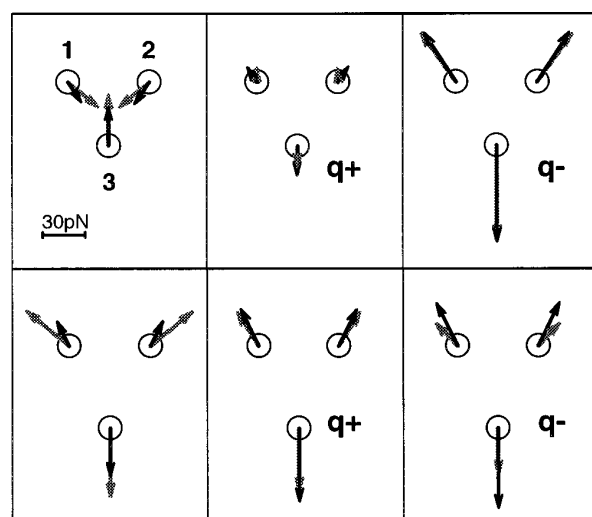

FIG. 2. Vectorial SIFs acting on three model solutes. Gray arrows, from MD simulations; black arrows, additively computed from pairwise data of Fig. 1. In all cases, 1 and 2 are electrically uncharged LJ spheres at 5 Å center-to-center distance. Top: $d_{1\text{-}3} = d_{2\text{-}3} = 4.6$ Å. Bottom: $d_{1\text{-}3} = d_{2\text{-}3} = 5.7$ Å. Solute 3 is, in turn, uncharged (left) or bearing a unitary electric charge, either positive (center) or negative (right).
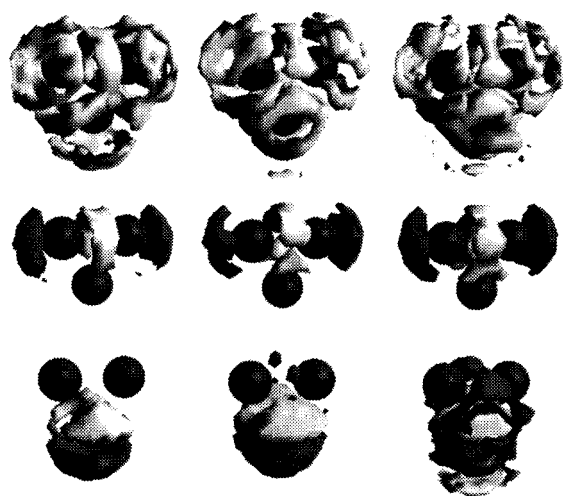
FIG. 3. Top: a 3D representation of hydration isoprobability surfaces, at $P = 1.55$ (as defined in text). Center: 3D representation of space-resolved contributions to the SIF component between solutes 1 and 2, along their center-to-center direction. Surfaces through elementary cells giving repulsive (white) and attractive (gray) contributions of 0.8 pN are shown. Bottom: as in center, for SIF on solute 3, surfaces cut at 0.25 pN. The solute configuration is as in Fig. 2, top. Solute 3 (from left to right) is uncharged, positively charged, negatively charged. Graphics from SciAn [24].

sign dependence of hydration patterns reflects the known different response of the aqueous medium to perturbations caused by opposite electric charges, due to the asymmetric charge distribution on water molecules [10,11]. In the center and bottom rows of the same figure we show the space resolved contributions to F_x (1-2) and F_y (3). Comparison of Figs. 2 and 3 shows that small (yet, easily detectable) changes of hydration cause large changes in SIFs. This is because (as also seen from the figure) SIFs are usually the result of the balance of opposite contributions, each of which exceeds by one or two orders of magnitude that of SIFs themselves [6,8]. It should be remarked that the observed strong dependence upon charge sign concerns also SIFs acting on uncharged LJ solutes, notwithstanding their being solely determined by the P distribution, regardless of the orientation of water molecules. This is not surprising if SIFs and hydration free energy are viewed in terms of perturbation of the configurational potential energy surface, or of inherent structures [6,7,18].

The present results elicit novel features of SIFs on the molecular scale, that is (i) a charge-sign dependent, repulsive contribution to SIFs between a pair of LJ solutes, generated when an electric charge is put on one of them, traceable to changes of hydration and of hydration overlaps and not amenable to continuum modeling of the solvent (ii) a strong and charge sign-dependent change of hydrophobic interactions between apolar solutes caused by a third, charged solute. These are strong (not second-order) modulation effects having a complex character, evidenced by their marked nonlinearity and charge sign and conflict dependence. The remarkable size of these modulations is shown, e.g., by Fig. 1 (top) and Fig. 2 (top) where a strong hydrophobic attraction between two apolar solutes is seen to be turned into an even stronger ''electrically induced hydrophobic repulsion.''

It is in order to note that the qualitative existence of hydrophobicity changes caused by charged solutes is experimentally known. Average effects of these changes, such as salting in or salting out have long been taken into account in terms of virial expansion. These and related large-scale and coarse-grained effects involving the averaging of a large number of interactions are expected [19] and found to be successfully treatable by computer efficient methods based on approximations or on continuum modeling of the solvent [9,20–22]. Such methods can adequately reproduce overall features of hydration and thermodynamic parameters [22]. However, as shown by the present results, SIFs on the microscopically detailed scale are determined by small differences of large terms. Their evaluation requires therefore details of hydration to a high degree of space resolution. At such resolution, charge-sign dependence, strong nonadditive modulation, and short-distance behavior of SIFs cause far from negligible effects. Consequently, the complementary MD approach taking into explicit consideration the fine grained system of individual solvent molecules and atoms becomes necessary [9]. This approach, which makes no use of truncations or of averaging outs is in particular necessary for revealing detailed features on which the exquisite specificity of intramolecular and intermolecular interactions of biomolecules rests.

Features of SIFs reported here add to and fully agree with other properties recently demonstrated on the molecular scale, all related to the strong many-body character of PMF in aqueous solutions. These are: marked nonadditivity [6] and context dependence [7], and extended range of action in complex solutes due to a nonlinear relay action of interposed solutes [8]. The strong dependence upon charge sign of effects elicited here agrees with and endorses results reported in Ref. [7], concerning the behavior of SIFs acting on residue side chains of a protein. Altogether, these properties are seen to be capable of causing even a sign reversal of SIFs, relative to expectations based on the sole character of the given solute element. The present results further elicit very strong, even qualitative modifications of hydrophobic SIFs caused by electric charges and showing a very sensitive dependence upon configurational context and charge sign. High configurational specificity is a key feature of intermolecular and intramolecular interactions of biomolecules. The relevance of SIFs to specificity is easily appreciated by noting that a typical SIF on a residue side-chain ($\sim$ 150 pN) performs, if displaced by 1 Å, a work equivalent to about 2 kcal mol^{-1}, which is of the order of stabilizing free energy of a protein. An important example of the biofunctional role of the strong modulability of hydrophobic forces by electric charges shown here might be found in the extreme specificity of binding domains for pTyr in signal transduction proteins, causing affinity modulations that span over three orders of magnitude [23]. More in general, it may concern the ubiquitous functional concomitance of protein charge and conformational changes.

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