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Classical Drude Polarizable Force Field Model for Methyl Phosphate and its Interactions with Mg^{2+}

Francesco Villa[†], Alexander D. MacKerell Jr.[‡], Benoît Roux[¶], and Thomas Simonson[†]

[†]Laboratoire de Biochimie (CNRS UMR7654), Ecole Polytechnique, Palaiseau, France

[‡]Department of Pharmaceutical Sciences, School of Pharmacy, University of Maryland, Baltimore, MD., USA

[¶]Department of Biochemistry and Molecular Biology, University of Chicago, Chicago and Biosciences Division, Argonne National Laboratory, Argonne, IL., USA

Abstract

Phosphate groups are essential components of nucleic acids and proteins, whose interactions with solvent, metal ions and ionic side chains help control folding and binding. Methyl phosphate (MP) represents a simple analog of phosphate moieties that are post-translation modifications in proteins and present at the termini of nucleic acids, among other environments. In the present study, we optimized parameters for use in polarizable molecular dynamics simulations of MP in its mono and dianionic forms, $\text{MP}^- \equiv \text{CH}_3\text{HPO}_4^-$ and $\text{MP}^{2-} \equiv \text{CH}_3\text{PO}_4^{2-}$, along with $\text{P}_i^{2-} \equiv \text{HPO}_4^{2-}$, in the context of the classical Drude oscillator model. Parameter optimization was done in a manner consistent with the remainder of the Drude molecular mechanics force field, choosing atomic charges and polarizabilities to reproduce molecular properties from quantum mechanics as well as experimental hydration free energies. Optimized parameters were similar to existing dimethyl phosphate parameters, with a few significant differences. The developed parameters were then used to compute magnesium binding affinities in aqueous solution, using alchemical molecular dynamics free energy simulations. Good agreement with experiment was obtained and outer sphere binding was shown to be predominant for MP^- and MP^{2-} .

Graphical abstract

Correspondence to: Thomas Simonson.

Supplementary Material

Supplementary Material is available providing more detailed data on the force field fitting: water energy scans, ion energy scans, conformational energies, vibrational modes.

1 Introduction

Phosphate groups are important in biochemistry.¹ Not only are they key nucleic acid components, they are also present in certain protein side chains, to which they are added/removed enzymatically as a function of cellular conditions. Often, the presence or absence of the phosphate group makes the protein competent or incompetent to bind another molecule.² For example, protein–protein interactions facilitated by phosphorylation are essential for a number of signaling pathways, so that understanding the atomic nature of such interactions can yield insights into a range of biological phenomena.³

Computer simulations can give detailed insights into the structure and interactions of phosphate groups. To exploit this capability it is essential to have accurate force field models of these moieties. However, developing accurate simulation models is challenging for ionic interactions, such as those involving phosphate groups. Simple, additive force fields^{4,5} are usually parameterized to describe ionic interactions between groups that are highly-exposed to aqueous solvent. When ionic interactions occur in solvent-poor environments, such as a protein–protein interface, additive force fields can give large errors.⁶ Indeed, the same group will usually be more polarized when it is exposed to solvent or close to an ion, and less polarized when it is removed from solvent or ions. Such effects require a force field that models electronic polarizability explicitly. Polarizable force fields have been parameterized for several ions in water, and used to calculate structures, dynamics, binding and solvation free energies.^{7–13} For example, dihydrogen phosphate H_2PO_4^- was studied recently with the AMOEBA force field and its Mg^{2+} binding free energy was computed through alchemical molecular dynamics (MD) free energy simulations,¹⁴ giving excellent agreement with experiment. Dimethyl phosphate (DMP) and its interactions with Mg^{2+} were studied with the polarizable Drude force field,¹⁵ giving good agreement with experiment for structure and dynamics.

Here, we extend the Drude polarizable force field to three other phosphate compounds and their interactions with Mg^{2+} . Methyl phosphate (MP) was studied in its protonated and deprotonated forms, $\text{MP}^- \equiv \text{CH}_3\text{HPO}_4^-$ and $\text{MP}^{2-} \equiv \text{CH}_3\text{PO}_4^{2-}$. While DMP is analogous to

the phosphate groups within DNA and RNA polymers, MP is analogous to the phosphate groups at DNA and RNA termini. MP can also be viewed as a fragment of phosphorylated side chains like phospho-serine or phospho-tyrosine. We also studied mono hydrogen phosphate, $\text{HPO}_4^{2-} \equiv \text{P}_i^{2-}$, for which experimental Mg^{2+} binding data is available. Drude force field parameters for MP^- , MP^{2-} , and P_i^{2-} were derived using the established methodology for the Drude force field.^{11,15–20} Their interactions with Mg^{2+} were described by extending slightly the model parameterized recently for DMP.¹⁵ This model takes advantage of the use of pair-specific Lennard-Jones (LJ) and through-space Thole scaling parameters, including LJ parameters on the water Drude particle- Mg^{2+} interactions and pair-specific phosphate- Mg^{2+} interaction parameters. To test the force field, we computed Mg^{2+} binding free energies using alchemical molecular dynamics (MD) free energy simulations. Alchemical calculations were performed using a double-decoupling method,^{21–23} where Mg^{2+} is reversibly deleted from aqueous solution in a series of MD simulations, then reintroduced into a complex with the phosphate ligand.

Mg –phosphate interactions are complex, as reviewed recently.^{24,25} Mg^{2+} can coordinate a single phosphate group or several at a time. It can coordinate each one directly (“Inner Sphere” or IS coordination) or through a water molecule (“Outer Sphere” or OS coordination). Inner Sphere coordination can be mono- or bidentate; i.e., with one or two coordinating oxygens. In addition, the phosphate can have several different protonation states, which may differ in IS and OS complexes. For MP^- and MP^{2-} , we considered both IS and OS coordination, and found that OS was predominant. For P_i^{2-} , we only considered OS coordination. The computed binding free energies were in good agreement with experiment.^{26,27} This contrasts with the CHARMM36 additive force field, which overestimated $\text{Mg}^{2+} - \text{P}_i^{2-}$ binding by an order of magnitude.²⁸ The results suggest that the Drude polarizable force field model will be useful to study the analogous phosphate groups in nucleic acids and phosphorylated protein side chains as well as for phosphate and related species in aqueous solutions, such as buffer systems.

2 Methods

2.1 Quantum mechanical calculations

We considered MP^- , MP^{2-} , and monohydrogen phosphate (HPO_4^{2-}) or P_i^{2-} . For each molecule, the molecular structure was optimized with Gaussian09²⁹ at the MP2/6-31G* level. Electrostatic potential (ESP) maps were computed at the same level, on ten concentric grids surrounding the molecule, totalling around 2000 points and ranging up to about 5 Å from the solute atoms. Around 65 perturbing charges of 0.5e were individually distributed around the molecule at selected grid points. An unperturbed map was computed, along with perturbed maps, each one in the presence of a single perturbing charge. The molecular polarizability was computed at the same level. The molecular dipole was computed at the RIMP2/6-31G* level using the PSI4 program.³⁰ The level of QM theory used for each property was chosen to be consistent with the past development of the Drude force field.

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We also considered each solute interacting with a single water molecule, positioned along a hydrogen bond donor or acceptor direction (see Table 2 inset). There were five directions for MP^- and four for MP^{2-} . The water–MP distance was varied from 1.6 to 3.0 Å in 0.1 Å steps, holding the geometry and orientation of both molecules fixed. For each distance, the MP–water interaction energy was computed at the RIMP2/cc-pVQZ level with Basis Set Superposition Error corrections³¹ using PSI4.

2.2 Drude model

For MP^- , MP^{2-} , and P_i^{2-} , Drude force field parameters were derived in this work. All atoms were polarizable except hydrogens. Two lone pairs were positioned on the hydroxyl oxygen O2 of MP^{2-} . For pairs of charges i, j (atoms or Drude pseudo-particles) that were covalently linked, or separated by two covalent bonds (1,3 pairs), Coulomb interactions were multiplied by the Thole screening function:³²

$$S(r_{ij}) = 1 - \left(1 + \frac{\tau_{ij} r_{ij}}{2(\alpha_i \alpha_j)^{1/6}} \right) \exp \left(-\frac{\tau_{ij} r_{ij}}{(\alpha_i \alpha_j)^{1/6}} \right) \quad (1)$$

This screening helps avoid overpolarization and instability, and helps optimize the molecular polarizability. Here, α_i, α_j are the atomic polarizabilities, r_{ij} is the interatom distance, and τ_{ij} is an adjustable parameter, which is the sum of atomic Thole scale factors, $\tau_{ij} = \tau_i + \tau_j$. Charges, polarizabilities, and Thole factors were assigned on an atomic basis, whereas Lennard-Jones (LJ) parameters and bonded parameters were assigned based on atom types. Atom types were mostly transferred from DMP. Exceptions were the MP^- hydroxyl group and the oxygen O1 that bridges the methyl and phosphate groups in MP^- and MP^{2-} . This atom was typed according to a phosphate from the Drude lipid force field.

2.3 Optimizing the Drude electrostatic parameters

We considered MP^- first. The parameters to fit were the atomic charges (q_i), polarizabilities (α_i), and Thole parameters (τ_i). The QM electrostatic potential maps were first fitted using the *drude-fitcharge* module of the GAAMP parameterization tool.¹⁷ Restraints were applied to the parameters to avoid large deviations from physically-reasonable reference values. The target function χ^2 to minimize was composed of several terms:

$$\chi^2 = \chi_{ESP}^2 + w_q \chi_q^2 + w_\alpha \chi_\alpha^2 + w_\tau \chi_\tau^2 \quad (2)$$

The first term is the mean quadratic difference between the QM and MM ESP maps; the other terms are weighted restraints. Reference values $\{q_i^0\}$ for charges were transferred from DMP and adjusted slightly by hand to match the net MP^- charge. The charge restraint term was

$$\chi_q^2 = \frac{1}{n} \sum_{i=1}^n f(q_i, q_i^0) \quad (3)$$

where n is the number of atoms and

$$f(q_i, q_i^0) = \begin{cases} 0 & |q_i - q_i^0| \leq 0.03 \\ (|q_i - q_i^0| - 0.03)^2 & \text{otherwise} \end{cases} \quad (4)$$

Polarizabilities α_i and Thole parameters τ_i were restrained quadratically. Reference polarizabilities were taken from Miller³³ then scaled by 0.7. Reference Thole factors were set to 1.3. Weights for the restraints were $w_q = 3.0$, $w_\alpha = 0.4$, $w_\tau = 0.2$ (the Drude defaults). We imposed two groups of equivalent atoms (Fig. 1): (H11, H12, H13) and (O3, O4), which shared the same charge, polarizability and Thole parameter. For the restrained electrostatic potential fit of MP^- , we further reduced the number of parameters by fixing some of the charges at their DMP⁻ values: $q(\text{H11}) = q(\text{H12}) = q(\text{H13}) = 0.026e$ and $q(\text{C1}) = 0.0725e$. For the O2 hydroxyl oxygen, the atomic charge was placed on the two associated lone pairs (as usual with Drude hydroxyls).

After fitting, the new MP^- electrostatic parameters better approximated the QM molecular dipole moment. In a second stage, we adjusted the atomic polarizabilities and Thole parameters by hand to fit the molecular polarizability. The target value is the QM value scaled by a factor of 0.85,¹⁸ a reduction shown to work well for the condensed phase. The MM polarizability was computed with the CHARMM program³⁴ by applying a uniform field along the x , y , and z directions (“pull efield” command). We fitted the diagonal components of the polarizability matrix and their sum, the total molecular polarizability. During adjustment, we cross-checked that the changes did not affect the ESP maps too much. The adjustments made the atomic polarizabilities and Thole values more like DMP.

In a third stage, we considered MP^- interactions with a single water molecule, placed along a hydrogen bond donor or acceptor direction. The water was described by the Drude polarizable SWM4-NDP model.³⁵ Atomic charges were then adjusted manually, keeping the other parameters fixed. Lennard-Jones (LJ) parameters were assigned according to existing atom types, taken from Drude DMP, except for the MP^- hydroxyl and the bridging oxygen O1 (Fig. 1). For the MP^- hydroxyl, we adopted the usual Drude hydroxyl types OD31A and HDP1A. For O1, we used the corresponding atom type from the Drude lipid phosphates.³⁶ After adjusting the charges, we checked the quality of the ESP maps, the dipole and the molecular polarizability.

For MP^{2-} , we used the polarizabilities and Thole factors obtained for MP^- and we optimized the atomic charges to fit all the QM data at once, through a grid search in charge space. We did an initial, coarse search then a finer search. For P_i^{2-} , we transferred parameters from

MP²⁻, then refined polarizabilities and Thole factors manually to fit the molecular polarizability. Finally, we refined charges through a grid search to fit the molecular dipole and water interaction energies.

2.4 Bonded parameters: QM and MM conformational energies

We borrowed bond/angle/dihedral parameters from the Drude DMP.¹⁵ Missing parameters for the MP⁻ hydroxyl were taken from MP⁻ in the CHARMM c36 additive force field.^{37,38} They included a harmonic Urey-Bradley term that restrains the P1–O2–H2 angle. We did vibrational analysis in Gaussian09 using MP2/aug-cc-pVTZ for structure optimization and normal mode calculations. The frequencies and normal modes were used to characterize the QM energies associated with small nuclear displacements. For each molecule (MP⁻, MP²⁻, DMP⁻), we generated 200 displacements by randomly combining small motions along all but the hydrogen stretching normal modes. The associated QM energies were computed by summing the contribution of each QM mode. For comparison to MM energies, we applied a scaling factor of 0.941 to the QM frequencies³⁹ before computing the QM displacement energies. We also computed MM normal modes with CHARMM and compared the QM and MM normal modes and conformational energies. Finally, we performed torsional scans of selected dihedral angles, computing MP2/6-31+G(d) QM and MM energies as a function of the torsion angle.

2.5 Magnesium binding model

Magnesium–water and magnesium–DMP interactions were parameterized recently for the Drude force field^{11,15} in the context of the SWM4-NDP Drude water model. The parameterization includes repulsive Lennard-Jones interactions between the Mg²⁺ ion and the Drude particles on the water oxygens (see Supplementary Material). For the Mg²⁺–MP and Mg²⁺ + P_i²⁻ Lennard-Jones interactions considered here, we mostly used the Mg²⁺ interaction parameters optimized for DMP. Two new parameters were introduced. For the MP⁻ hydroxyl oxygen O2, we introduced a specific Thole scaling factor for its Mg²⁺ interaction (see NBTHOLE entry in Supplementary Material). This value was chosen to fit the MP⁻–Mg²⁺ interaction energy from QM as a function of the Mg²⁺ distance along the direction of the oxygen's pairs. For the bridging oxygen O1 between the phosphate and the methyl of MP⁻ and MP²⁻ (Fig. 1), we introduced specific Lennard-Jones parameters (see NBFIX entry in Supplementary Material). The values were chosen to fit the MP⁻–Mg²⁺ interaction energy from QM along the direction that bisects the O1–P1 and O1–C1 covalent bonds (in the direction pointing away from the C1–O1–P1 triangle, not into it).

For both MP⁻ and MP²⁻, additional adjustments were made to several Thole factors in order to eliminate large excursions by some of the Drude particles away from their host atom during MD simulations in complex with Mg²⁺. The Thole values for 2–3 atoms were altered by 0.1–0.2. These adjustments had a very small effect on the QM/MM agreement.

2.6 Alchemical free energy calculations

To obtain the Mg²⁺–MP and Mg²⁺ + P_i²⁻ binding free energies, we simulated the alchemical annihilation or “decoupling” of Mg²⁺ alone in solution and bound to the phosphate solute,

over a series of MD simulations.^{21–23} For MP^- , we considered Outer Sphere (OS) binding (Mg^{2+} is separated from the phosphate by a bridging water molecule, see Fig. 4), which was shown earlier to predominate.¹⁴ For P_i^{2-} , we also considered OS binding. For MP^{2-} , we considered both OS and Inner Sphere binding (IS; Mg^{2+} directly coordinates the phosphate). Decoupling was done in two stages. First, the Mg^{2+} charge and polarizability (its Drude charge) were gradually reduced to zero. They were scaled by a coupling parameter λ_q which took the successive values 1, 0.9, ..., 0.1, 0. For each value, an MD simulation was performed. In the second stage, the Lennard-Jones interactions of the uncharged Mg^{2+} were gradually removed. MD was done with an energy function that depended on a coupling parameter λ_{LJ} , such that when $\lambda_{LJ} = 1$, the Lennard-Jones interactions were fully present, and when $\lambda_{LJ} = 0$, they were reduced to zero (Mg^{2+} was decoupled). We did simulations with $\lambda_{LJ} = 1, 0.9, 0.8, 0.6, 0.4, 0.2$, and 0. The Mg^{2+} LJ term had a soft-core form,⁴⁰ to ensure a gradual, singularity-free decoupling. Each solute was solvated by 1000 SWM4-NDP water molecules. MD was done at room temperature and pressure, using Langevin dynamics with a Langevin Piston Nosé-Hoover barostat.^{41,42} The simulations used periodic boundary conditions with a cubic box of edge length $L \approx 31 \text{ \AA}$, and a Particle Mesh Ewald electrostatic treatment (PME). Bonds to hydrogen atoms were constrained with the SHAKE method.⁴³ LJ interactions were shifted to zero for separations between 10 and 12 $\text{\AA}$. The MD timestep was 1 fs. Each MD simulation lasted 3 ns, so that a full decoupling calculation used 51 ns of MD. Simulations were done with the NAMD program.^{44,45} For the LJ decoupling, free energy changes between successive λ_{LJ} values were computed using Bennett's Acceptance Ratio or "BAR" method,⁴⁶ implemented in a perl script. For the electrostatic decoupling, the free energy change was obtained with thermodynamic integration, or "TI".⁴⁷ The free energy derivative was estimated at each λ_q value with a finite-difference approximation;²⁸ the derivative values were interpolated with cubic splines and integrated.

We emphasize that although the Drude model uses two distinct thermostats, TI is formally correct and easy to apply in the limit of a very cold Drude thermostat.⁴⁷ Indeed, we computed the energy derivative U/λ by a finite-difference approximation, which involved calculating the system energy for a series of snapshots produced with the energy function $U(\lambda)$. The energy was computed once with the energy function $U(\lambda)$ and once with the perturbed energy function $U(\lambda + \delta\lambda)$. In principle, when using $U(\lambda + \delta\lambda)$, the positions of the Drude particles within each snapshot should be adjusted to reflect the altered coupling constant. However, in the low-temperature limit, the Drude particles arrange themselves so as to minimize the system energy. Therefore, $U(\lambda + \delta\lambda)$ is not sensitive to a slight change in Drude positions, such as the one associated with the small λ increment. The associated energy error scales with λ as $\delta\lambda^2$, and the finite-difference derivative computed this way (without adjusting the Drude positions) is accurate.

Throughout the simulations, the MP or P_i^{2-} center of mass was harmonically restrained to stay near the box center. A second restraint was applied to the Mg^{2+} -P distance, which held it within a specific range $[a, b]$. Outside this range, the restraint energy increased harmonically with a force constant of $c = 4$ or $8 \text{ kcal/mol/\AA}^2$. This restraint had very little effect when Mg^{2+} was fully-coupled, but prevented it from wandering away when

decoupled. The free energy contribution of the restraint was computed analytically (see below).

2.7 Obtaining the binding free energy

To obtain the binding free energy G_{bind} , we started from an ideal-dilute, $\rho^\circ = 1$ M standard state solution of $\text{Mg}^{2+} + \text{MP}$. We used a thermodynamic cycle (Fig. 2) that decomposes the binding reaction into the following six steps. (1) We changed the concentration to a very low value, ρ^∞ . The corresponding free energy change was $-2kT \ln \rho^\infty / \rho^\circ$. (2) We removed the Mg^{2+} charge and LJ interactions alchemically. (3) We introduced the flat-bottomed harmonic restraint that keeps MP close to Mg. The restraint free energy includes a term $kT \ln \rho^\infty$, plus concentration-independent terms (see below). (4) We reintroduced the Mg^{2+} charge and Lennard-Jones interactions. (5) We restored the standard state concentration and (6) removed the restraint. The last restraint contribution was negligible, while the concentration change gave a free energy contribution $kT \ln \rho^\infty / \rho^\circ$. Overall, the ρ^∞ contributions cancel out and the binding free energy depends on concentration through a single, $kT \ln \rho^\circ$ term, as expected.⁴⁸

The charging free energies (steps 2, 4) were obtained by simulating the charging processes in a series of MD simulations at a finite concentration (one solute per MD box of edge length L). The simulations led to a free energy change $G(L)$. We deduced the value at the concentration ρ^∞ by extrapolating to the limit of a very large box. We used a theoretical treatment that accounts for electrostatic interactions of the Mg^{2+} with surrounding boxes and with the neutralizing gellium associated with PME.^{49–52} For a spherical ion of radius R , with a point charge q at its center, solvated in a cubic box of edge length L , the large-box charging free energy has the form:

$$\Delta G(L \rightarrow \infty) = \Delta G(L) + \frac{q^2 |\zeta|}{2\epsilon_W L} + \frac{2\pi q^2 R^2 \epsilon_W - 1}{3L^3 \epsilon_W} + O(R^2 L^{-3} \epsilon_W^{-3}) \quad (5)$$

Here, $\zeta = -2.837297$ is a constant and ϵ_W is the dielectric constant of the solvent. The free energy G corresponds to charge *insertion*. For the Mg^{2+} radius, we used $R = 1.2$ Å when Mg^{2+} was unbound and $R = 1.5$ Å when it was bound.

The free energy to apply the flat-bottomed harmonic restraint (step 3) was computed analytically. The restraint had the form:

$$U_R(r) = \begin{cases} \frac{1}{2}c(r-a)^2 & r < a \\ \frac{1}{2}c(r-b)^2 & r > b \\ 0 & \text{otherwise} \end{cases} \quad (6)$$

Here, r is the Mg–P distance, a and b define the allowed distance range, and $c = 4$ or 8 kcal/mol/Å² is the force constant. The flat-bottomed interval $[a, b]$ was 0.0 – 3.5 Å (IS complex) or 4.1 – 5.9 Å (OS complex). The free energy to remove the restraint in the presence of Mg^{2+}

was neglected, since the fully-charged Mg^{2+} stays in the corresponding range without any restraints and the restraint energy was tiny (less than 0.05 kcal/mol). The free energy to introduce the restraint when Mg is uncharged has the form:

$$\Delta G_{\text{rest}} = kT \ln \rho Q(c), \quad (7)$$

where ρ is the concentration and

$$\begin{aligned} Q(c) &\approx \int_0^\infty 4\pi r^2 \exp[-\beta U_R(r)] dr \\ &= \int_0^a 4\pi r^2 \exp\left[-\frac{\beta c}{2}(r-a)^2\right] dr + \frac{4}{3}\pi(b^3 - a^3) + \left(\frac{2\pi kT}{c}\right)^{3/2} + \frac{8\pi kTb}{c} + (2\pi)^{3/2}b^2\sqrt{\frac{kT}{c}} \end{aligned} \quad (8)$$

In practice, we computed the restraint free energy at the standard state concentration ($\rho = \rho^\circ = 1\text{M} = 11.84 \times 10^{-3} \text{ \AA}^{-3}$), which leads to the correct overall concentration dependence of the binding free energy.

3 Results

3.1 Fitting the force field parameters to QM target data

3.1.1 MP^- electrostatic parameters—The fit to MP^- ESP maps was characterized by a target function of $\chi^2 = 0.90$, with $\chi_{\text{ESP}}^2 = 0.80$, which improved over an initial value of $\chi_{\text{ESP}}^2 = 10$ associated with the initial guess parameters. The MP^- polarizability and dipole moment were well-reproduced (Table 1) by transferring parameters from DMP and modifying the O1 Thole value. We also computed MP^- –water interaction energies for waters located along hydrogen bond donor/acceptor directions (Table 2). The interaction with the O1 acceptor was poorly fitted, initially. Therefore, we replaced the original O1 atom type (OD30BN, from DMP) with a new type (OD30), similar to a Drude lipid phosphate (OD30B). We then performed charge adjustment manually to fit the QM interaction energies. Two of the Thole factors were also adjusted, to avoid large excursions of the Drude particles in MD simulations (see Methods). Table 2 reports QM and MM water interaction energies e_{min} at the minimum distance r_{min} for the MP^- acceptor and donor atoms. Full radial energy scans are shown in Supplementary Material. We also performed energy scans for the ions Na^+ and Mg^{2+} along the same directions. Agreement was satisfactory after adjusting interaction parameters for the bridging oxygen O1 of MP^{2-} and MP^- (Fig. 1) and the hydroxyl oxygen O2 of MP^- . The interaction energies are in Supplementary Material.

3.1.2 MP^{2-} and P_i^{2-} electrostatic parameters—For MP^{2-} , initial polarizabilities and Thole values were taken from MP^- . Polarizabilities were then adjusted slightly to better fit

the molecular polarizability (scaled QM value). Charges were then optimized to fit all the QM data at once, using a two-step grid search, where the ranges explored (in units of e) were 1.01:1.21 (P), -0.92: -0.72 (O2), -0.20:0.20 (C), and 0.02:0.09 (H). Two Thole factors were adjusted to eliminate large excursions of the Drude particle during MD (see Methods). The final value of the target function for the electrostatic potential maps was 2.37, about 3 times the MP^- value, consistent with the larger MP^{2-} charge. The polarizability, dipole moment, and water interactions were well-reproduced by the final model (Tables 1, 2).

For P_i^{2-} , initial parameters were transferred from MP^{2-} . Polarizabilities and Thole factors were adjusted manually to fit the scaled molecular polarizability. This included changing the hydroxyl polarizability to be isotropic (see details in Supplementary Material). Charges were then optimized through a grid search to fit the molecular dipole and water interaction energies. Results are included in Tables 1 and 2.

3.1.3 Conformational energies—The QM and MM minimum energy conformations of MP were in good agreement (Table 3). The largest deviation was for the O1–P1 bond length in MP^{2-} , which was 1.78 Å in the QM structure but 1.58 Å in the MM structure. The MM bond parameters were chosen to match the CHARMM36 additive force field for MP^{2-} ,⁵³ dinegative terminal phosphates in DNA,^{37,38} and dinegative phosphotyrosine.⁵⁴ They are also consistent with experimental X-ray structures of small phosphate compounds.⁵³ We performed dihedral energy scans for MP^- and MP^{2-} that explored the torsions involving the methyl and hydroxyl groups. The QM and MM energies are compared in Fig. 3. Good agreement was obtained with torsion parameters borrowed from DMP, with one exception. For the methyl rotation angle (C1–O1–P1–O2), the DMP parameters gave poor agreement, but good agreement was obtained using torsion parameters from the Drude DNA force field;¹⁹ see Supplementary Material.

For MP^- and MP^{2-} , we also generated 200 small displacements by randomly combining small motions along the quantum mechanical normal modes. The associated QM energies were computed by summing the contribution of each QM mode (using scaled frequencies; see Methods). Normal mode calculations were also done for DMP, as a reference. QM and MM energies are compared for each molecule in Supplementary Material. MM and QM energies and normal modes were in good agreement.

3.2 Mg^{2+} binding free energies

Experimental Mg^{2+} binding free energies are available for HPO_4^{2-} (P_i^{2-}) and H_2PO_4^- (P_i^-),^{26,27} but not for methyl phosphate. Nevertheless, we expect that the methyl substitution has a modest effect on the free energy, since Mg^{2+} binds on the opposite side of the molecule, as shown in Fig. 4. This is further supported by the similar OS binding free energies computed below for MP^{2-} and P_i^{2-} , within 1.2 kcal/mol of each other. Therefore, comparison to the experimental values should provide a valid test of the MP force field model. For MP^- and MP^{2-} , we considered both IS and OS binding. The IS and OS binding modes are shown in Fig. 4 using snapshots from the MD simulations. The MP^- IS complex mostly coordinated Mg^{2+} through a single oxygen (mean Mg–O distance of 2.07 ± 0.05 Å). The MP^{2-} IS

complex mostly had bifurcated Mg^{2+} coordination, with two of the phosphate oxygens interacting closely with Mg^{2+} (mean distances of 2.09 ± 0.07 Å). The IS complexes were stable during 1 ns of unrestrained MD, indicating that they are separated from OS by a free energy barrier. The MP^{2-} OS complex was also stable during 1 ns of MD, with no tendency to unbind or shift to IS. The MP^- OS complex had a shorter lifetime, estimated from a 10 ns unrestrained MD simulation. At the simulation concentration (55 mM), the MP^- OS binding free energy was 0.4 kcal/mol (see below), giving an expected long-time limit of 33% for the OS population. The observed population during the 10 ns of MD was 27%, indicating that rough convergence was achieved for the binding/unbinding rates (which is sufficient for our purpose). The complex remained bound for intervals of about 275 ps on average, rebinding a few hundred picoseconds later. Evidently, the barrier separating the OS bound and unbound states is small, probably on the order of 2 kcal/mol. Transitions to IS were not observed. The OS lifetime is roughly comparable to the rate of OS/unbound transitions obtained for P_i^- with the Amoeba force field (which was not measured precisely).¹⁴

For P_i^{2-} , we only considered OS binding, for several reasons. OS binding was predominant for MP^{2-} in the present simulations, see below; we expect P_i^{2-} to behave similarly. It also proved difficult in this work to obtain a good force field model for short-range $\text{P}_i^{2-} - \text{Mg}^{2+}$ interactions using the same van der Waals parameters as for MP^{2-} . More extensive parameter optimization with one or two new atom types would be necessary. Since modeling inorganic phosphate was not our goal in this work, we left a full parameter optimization and IS binding study for future work.

The standard state binding reaction was decomposed into six steps (Fig. 2). Step 1 changed the concentration from the standard state value $\rho^\circ = 1$ M to a very low value ρ^∞ (near-infinite dilution); step 5 did the reverse. Overall, the ρ^∞ contributions cancel out and the binding free energy includes only a $kT \ln \rho^\circ$ concentration dependence, as expected. In practice, to respect this form, we computed the restraint free energy at the standard state concentration. The free energies to remove, then restore the Mg^{2+} charge and Lennard-Jones interactions were computed alchemically (double-decoupling method^{21–23}). The MD values were extrapolated to the infinite-dilution limit analytically (see Eq. 5). The calculation was done for each complex and for unbound Mg^{2+} . The computed free energies and their components are given in Table 4. The unbound Mg^{2+} decoupling free energy, 412.3 kcal/mol, is 1.8 kcal/mol larger than the result (corrected for box size effects; Eq. 5) of earlier simulations¹⁵ that used a smaller model and shorter runs. Repeating the entire decoupling calculation (51 ns) for the Mg^{2+} – MP^- complex gave the same result within 0.1 kcal/mol, indicating good convergence.

For MP^- , the computed binding free energies were –2.0 kcal/mol for the OS state, 0.3 kcal/mol for the IS state, and –2.0 kcal/mol overall. All three values are within 0.8 kcal/mol of the AMOEBA predictions for H_2PO_4^- .¹⁴ The overall binding free energy is 0.3 kcal/mol away from the experimental H_2PO_4^- value of –1.7 kcal/mol.²⁶ Binding is predicted to be dominated by the OS state, which has a 97% occupancy, close to the AMOEBA H_2PO_4^-

prediction (95% OS).¹⁴ For MP^{2-} , computed binding free energies were -2.6 kcal/mol for the OS state, -1.5 kcal/mol for the IS state, and -2.7 kcal/mol overall. The overall value is 1.0 kcal/mol away from the experimental HPO_4^{2-} value of 22 ± 3.7 kcal/mol.²⁷ The bound state is predicted to be 87% OS and 13% IS. Earlier *ab initio* calculations with a dielectric continuum solvent also predicted that OS was favored over IS, but with a smaller difference.²⁸ For P_i^{2-} , the OS binding free energy was -4.0 kcal/mol, close to the experimental value. The OS values for MP^{2-} and P_i^{2-} show that the methyl substitution has a modest effect, as expected, weakening binding by 1.3 kcal/mol. We note that for MP_2^- , HPO_4^{2-} , and unbound Mg^{2+} , the correction for the MD box size was small but distinctly non-negligible (over 1 kcal/mol). It was zero for MP^- , since introducing Mg^{2+} changes the total charge from -1 to $+1$ (same absolute charge magnitude).

Overall, for all three complexes considered, the computed binding free energies are in good agreement with experiment. This contrasts dramatically with the additive, CHARMM36 force field, which overestimated the MP^{2-} binding free energy by an order of magnitude.²⁸ The results are consistent with mostly OS binding for all the complexes.

4 Conclusions

Methyl phosphate can be seen as an analog of groups present in nucleic acids and phosphorylated amino acid side chains. Its Mg^{2+} binding gives insight into Mg^{2+} binding by these molecules. For this and similar binding processes, atomistic simulations are a powerful tool. However, additive force fields have given large errors for phosphate- Mg^{2+} binding free energies²⁸ and for several other ion-ion interactions.⁶ Here, we developed parameters consistent with the Drude polarizable force field, which provides a simple, explicit description of electronic polarizability. Atomic charges, polarizabilities, and Thole scale factors were optimized to reproduce QM data. The parameters obtained were similar to those derived earlier for DMP,¹⁵ with some significant differences. Agreement between the QM and MM data was similar to that obtained earlier for DMP. In addition to MP^- and MP^{2-} , we parameterized P_i^{2-} . The force field model is not yet complete, since the P_i^{2-} parameters do not accurately describe its short-range interactions with divalent Mg^{2+} . Such interactions are not relevant for phosphorylated amino acid side chains.

For MP^- and MP^{2-} , we considered both IS and OS Mg^{2+} binding (Fig. 4). We found that OS conformations represented over 85% of the bound state, while IS conformations made a small contribution to the binding free energy. The IS conformations mostly displayed Mg^{2+} coordination by one (MP^-) or two (MP^{2-}) phosphate oxygens. The computed binding free energies agreed well with the experimental values for P_i^- and P_i^{2-} , respectively. The MP^{2-} OS value was 1.4 kcal/mol less negative than the value computed for P_i^{2-} . Thus, the methyl substitution had a moderate effect on Mg^{2+} binding. Free energy simulations were performed earlier for the monoanionic P_i^- , based on the AMOEBA polarizable force field.¹⁴ They gave close agreement with experiment for the Mg^{2+} binding free energy and showed

that OS conformations represent 95% of the bound state. From these results, we expect that OS binding also predominates for P_i^{2-} . Indeed, our computed $P_i^{2-} - Mg^{2+}$ OS binding free energy agreed closely with the experimental value for P_i^{2-} . We conclude that the Drude model accurately describes MP and Mg^{2+} -MP binding. We also conclude that OS binding is predominant for all four phosphate compounds: MP^{2-} , MP^- , P_i^{2-} and P_i^- . This is the first time the predominant bound state has been clearly identified for MP^{2-} , and MP^- .

The parametrization and simulation of MP with the Drude polarizable force field is a step towards modeling phosphorylated side chains in proteins. The Drude force field is applicable to large biomolecules and provides the speed and accuracy for long and wellconverged simulations.^{6,19,55–57} It can be used with the programs NAMD,⁵⁵ GROMACS⁵⁸ and OpenMM⁵⁹ as well as CHARMM.³⁴ The accuracy obtained here for phosphate– Mg^{2+} binding indicates that the Drude force field can be applied to elucidate molecular recognition processes involving phosphorylated proteins, which are essential for biological signalling.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.
Phosphate compounds parameterized in this work.

Figure 2. Mg^{2+} -methyl phosphate (MP) binding reaction (left) decomposed into steps.

Figure 3.
QM and MM torsion energies.

Figure 4.
 Mg^{2+} -phosphate binding conformations from MD simulations. Selected waters are shown (not the full Mg^{2+} coordination sphere).

Table 1

MP²⁻, MP²⁻ and P_t²⁻ molecular polarizabilities and dipole moments

molecule	method	polarizability (Å ³)				dipole (D)			
		α_{xx}	α_{yy}	α_{zz}	α	μ_x	μ_y	μ_z	μ
MP ⁻	QM	8.28	7.26	6.92	7.49	-4.24	2.54	-1.06	5.06
	QM×0.85	7.04	6.17	5.88	6.36	-2.74	1.62	-0.57	3.23
	MM	7.09	5.96	5.88	6.31				
MP ²⁻	QM	9.83	7.80	7.92	7.49	-4.57	-0.45	0.01	4.59
	QM×0.85	8.36	6.63	6.73	7.24	-4.23	0.47	0.00	4.26
	MM	7.59	6.50	6.49	6.86				
P _t ²⁻	QM	7.05	6.72	6.69	6.82	0.12	-1.10	-2.36	2.61
	QM×0.85	5.99	5.71	5.68	5.80	0.08	-0.67	-1.10	1.29
	MM	4.75	4.80	4.47	4.67				

MP–water or Mg^{2+} and P_i^{2-} –water interaction distances r_{min} (Å) and energies e_{min} (kcal/mol) at the minimum energy separation. Full results for Mg^{2+} and Na^+ are in Supplementary Material.

Table 2

molecule	probe	atom	QM		MM	
			r_{min}	e_{min}	r_{min}	e_{min}
MP [−]	H ₂ O	O1	1.9	−11.66	1.8	−11.40
	H ₂ O	O2	1.9	−10.58	1.8	−10.93
	H ₂ O	O3	1.8	−13.43	1.7	−13.66
	H ₂ O	O4	1.8	−12.93	1.7	−13.93
	H ₂ O	H	2.0	0.07	2.0	0.55
	Mg ²⁺	O2	1.9	−258.81	1.8	−258.79
MP ^{2−}	H ₂ O	O1	1.7	−21.08	1.7	−21.18
	H ₂ O	O2	1.6	−22.75	1.6	−22.70
	H ₂ O	O3	1.6	−22.88	1.6	−22.80
P _i ^{2−}	H ₂ O	O1	1.7	−20.77	1.6	−21.00
	H ₂ O	O2	1.6	−22.41	1.6	−23.15
	H ₂ O	O3	1.6	−22.61	1.6	−22.62

Table 3Comparing QM and MM structures of MP⁻ and MP²⁻

	MP ⁻			MP ²⁻			
	QM	MM	diff.	QM	MM	diff.	
bonds (Å)	C1-O1	1.43	1.44	0.01	1.41	1.43	0.03
	O1-P1	1.68	1.59	0.09	1.78	1.58	0.20
	P1-O2	1.69	1.58	0.11	1.55	1.50	0.06
	P1-O3	1.51	1.49	0.02	1.45	1.49	0.04
	P1-O4	1.51	1.49	0.02	1.56	1.50	0.06
O2-H2	0.97	0.97	0			0	
angles (°)	C1-O1-P1	115.7	119.1	3.4	113.3	116.8	3.5
	O1-P1-O2	99.9	99.6	0.3	102.8	102.8	0
	O1-P1-O3	105.6	110.3	4.7	100.9	103.4	2.5
	O1-P1-O4	108.4	110.6	2.2	102.7	102.8	0.1
	P1-O2-H2	105.3	103.9	1.4			0
torsions (°)	C1-O1-P1-O2	71.3	76.4	5.1	59.4	59.7	0.3
	C1-O1-P1-O3	177.5	-177.4	0.1	180.0	180.9	0.9
	C1-O1-P1-O4	-39.9	-36.7	3.2	-59.5	-59.6	0.1
	O1-P1-O2-H2	97.4	99.3	1.9			0

Table 4

Mg²⁺-phosphate binding free energies from simulations and experiment

solute or complex	$G_{\text{dec}} (\rho_{\text{MD}})$	size correction	restore LJ	G_{LJ}	^a apply restraint	^b G_{bind}	Expt. ^c
Mg ²⁺ ;MP ⁻ (OS)	-415.6	0.0	+0.8		+0.4	-414.4	-1.7
Mg ²⁺ ;MP ⁻ (IS)	-414.4	0.0	+1.0		+1.1	-412.3	-1.7
Mg ²⁺ ;MP ²⁻ (OS)	-417.5	+1.0	+1.0		+0.4	-415.1	-3.7
Mg ²⁺ ;MP ²⁻ (IS)	-416.5	+1.0	+1.6		+1.1	-412.8	-1.5
Mg ²⁺ ;P _i ²⁻ (OS)	-418.5	+1.0	+0.8		+0.4	-416.3	-3.7
Mg ²⁺	-414.1	+0.9	+0.9		-	-412.3	-

In kcal/mol.

^a At the standard state concentration.

^b Obtained by subtracting the Mg²⁺ value from the value in "total" column.

^c Values are for H₂PO₄⁻ ≡ P_i⁻ and HPO₄²⁻ ≡ P_i²⁻, 26.27