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INTERMOLECULAR CHELATION OF TWO SERINE PHOSPHATES BY Ca^{2+} AND Mg^{2+}

A THEORETICAL STRUCTURAL INVESTIGATION

NOHAD GRESH

Institut de Biologie Physico-Chimique, Laboratoire de Biochimie Théorique, 13 Rue P. et M. Curie, 75005 Paris (France)

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Summary

The modes of interaction of Ca^{2+} and Mg^{2+} with 11 pre-selected conformations of serine phosphate (SP) are investigated by using an additive procedure based on ab initio Self Consistent Field computations for the calculation of intermolecular interaction energies. Possible models for the arrangements, SP-Ca^{2+}-SP and SP-Mg^{2+}-SP, are investigated.

The comparison between the binding energetics of Mg^{2+} and Ca^{2+} to one and two serine phosphates is discussed. It appears that some specific arrangements, SP-M^{2+}-SP (M^{2+} = Ca^{2+} or Mg^{2+}), are able to account for the displayed marked selectivity of phosphatidylserine for Ca^{2+}, in keeping with the distinctive features of this complex in model membranes.

Introduction

The interaction of phosphatidylserine (PS) with Ca^{2+} has been the object of extensive studies carried out on model membranes [1—14]. It is responsible for an induced shift in the phase transition in phosphatidylserine membranes [6,8,13] and lateral phase separation in mixed phosphatidylcholine (PC)-phosphatidylserine membranes [7,8,13]. It promotes aggregation and fusion of phosphatidylserine vesicles [11,14]. These phenomena, which may have far-reaching biological implications, have been attributed to the formation of a PS-Ca^{2+} precipitate of unelucidated structure, having little or no removable water [7,9,12], and in which the hydrocarbon chains are in a crystalline state [8,11,12] and display a tight degree of packing [12].

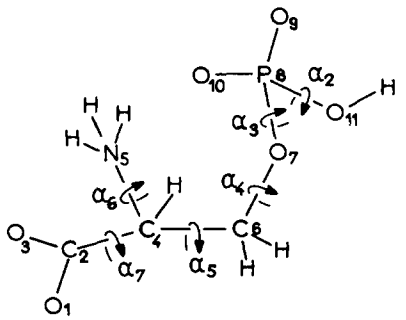

Fig. 1. The molecule of serine phosphate: atom numbering and torsion angles.

On the other hand, Mg^{2+} has a much smaller binding constant for phosphatidylserine than Ca^{2+} [3,12], is ineffective in causing either phosphatidylserine vesicle fusion [11,14] or phase separation in mixed PC-PS membranes [7,8], and the PS-Mg^{2+} salt retains a considerable amount of water [12].

As a first step towards an understanding of the elements involved in the above-mentioned phenomena, we have performed theoretical computations of the intermolecular interaction energy of Ca^{2+} with one serine phosphate molecule, the anionic part of the polar head of phosphatidylserine, and of different possible modes of chelation of two serine phosphates by Ca^{2+}. The molecular structure of serine phosphate is given in Fig. 1.

Computations involving Mg^{2+} and this ligand have also been performed in a limited number of cases, in order to differentiate between the preferred modes of interaction of this cation with the ligand with respect to the binding of Ca^{2+}.

Procedure

The intermolecular interaction energies are computed with the use of an empirical procedure, elaborated in this laboratory, which has been shown to reproduce fairly satisfactorily the results of ab initio Self Consistent Field computations of such interactions [15]. It was abundantly shown that the SCF method, utilized in the supermolecule approach, can provide reliable results for such computations when appropriate basis sets are used (see, e.g., Ref. 16) and this method was used already in a study of the intrinsically preferred modes of interaction of Na^+ with serine phosphate [17]. Its use is, however, precluded for the present work, owing to the large dimensions of the supersystems investigated.

The interaction energies consist of a sum of electrostatic, polarization, repulsion and dispersion contributions. The electrostatic contribution is computed through the use of a multipolar expansion of the self consistent field wave functions of the corresponding monomers [18,19]. This expansion positions monopoles (point charges), dipoles and quadrupoles on every individual atom of a considered molecule as well as on the middle of every atom-pair, the dipoles and quadrupoles pertaining to non-bonded atoms not being retained;

the electrostatic component is then computed as a sum of multipole-multipole interactions between the envisaged molecules. The polarization contribution is computed by using a multipolar expansion of the electrostatic field generated by every individual molecule in the supersystem considered, and by using experimental bond polarizabilities, partitioned consistently into pure atomic and pure bond contributions. Intervening in the expression of the dispersion and repulsion contributions are empirical parameters, $K(i,j)$, characterizing the pairs of interacting atoms, i and j, from the involved molecules. The values adopted for Mg^{2+} and Ca^{2+} are the same as those used for Na^+ and K^+, respectively. The effective radii of the cations are 1.485 and 1.725 Å, respectively. These parameters were calibrated on the systems, Mg^{2+}-H_2O and Ca^{2+}-H_2O, so as to reproduce the self consistent field values of Ref. 16 (computations IIB).

The reliability of the procedure is assessed in Table I, in which are reported the results of a comparison between ab initio self consistent field and empirical results of computations on the interaction of Ca^{2+} with H_2O, formate and dimethylphosphate. The self consistent field computations were performed with the basis set of Ref. 20 and the atomic and bond multipoles were accordingly derived from the wave functions of the individual monomers computed with the same sets.

The numerical agreement between this method and the empirical procedure is satisfactory. The relative ordering of the conformers of dimethylphosphate

TABLE I

A COMPARISON BETWEEN AB INITIO SELF CONSISTENT FIELD AND EMPIRICAL RESULTS FOR THE INTERACTIONS: Ca^{2+}-H_2O, Ca^{2+}-FORMATE AND Ca^{2+}-DIMETHYLPHOSPHATE DISTANCES IN Å; ENERGIES IN KCAL/MOL.

The notations, *gg*, *gt* and *tt* indicate the usual [20] *gauche-gauche*, *gauche-trans* and *trans-trans* conformations of dimethylphosphate. B is the bridge position bisecting the OPO^- (or OCO^- in formate) angle, E is the external position in the same plane, at θ = P—O—Ca^{2+} = C—O—Ca^{2+} = 120° below the PO axis in dimethylphosphate$^-$ and the CO axis in formate.

Interaction	Self consistent field values		Empirical values	
	d	ΔE	*d*	ΔE
Ca^{2+}-H_2O	2.30	−50.9	2.30	−48.6
Ca^{2+}-formate				
B site	2.25	−281.1	2.35	−271.5
E site	2.05	−238.0	2.05	−233.4
Ca^{2+}-dimethylphosphate				
B site				
Conformer				
gg	2.30	−285.0	2.25	−272.4
gt	2.30	−280.3	2.25	−270.1
tt	2.30	−275.2	2.25	−266.8
Ca^{2+}-dimethylphosphate				
E site				
Conformer				
gg	2.15	−227.0	2.05	−227.1
gt	2.15	−220.3	2.05	−223.6
tg	2.15	−240.0	2.05	−238.6
tt	2.15	−233.5	2.05	−234.4

upon binding of Ca^{2+}, as well as the trend upon its binding to the formate, are correctly reproduced by the empirical procedure.

Self consistent field computations have been performed for 11 selected conformations of serine phosphate so as to obtain the multipole development necessary for the interaction computations. As in Ref. 17, minimal Gaussian orbital basis sets are used and the effect of the atomic core electrons is replaced by adapted pseudopotentials, according to the method developped by Melius, Goddard and Kahn [21—23]. The analytical expressions for the effective potentials and the parameters for the atoms from the first to the third row are those derived by Melius [23] and Topiol et al. [24]. The basis sets used for the valence electrons of C, N, O and P correspond to the least-squares fit set discussed in Ref. 25 and contracted into minimal. The basis set used on the hydrogens is the same as in Ref. 20, the d*zeta* exponent of the methylene and hydroxyl hydrogens being 1.2, and that of the ammonium hydrogens, 1.5 (see Ref. 16).

Throughout this study, the phosphate, carboxylate and ammonium groups are assumed to be ionized, which is their state prevailing at neutral pH [26,40].

The adopted bond lengths and valence angles of serine phosphate are taken from a crystallographic study of L-*o*-serine phosphate [27], with the following exceptions: the two bond lengths, $C\text{-}O^{\delta-}$, are taken to be both equal to 1.25 Å, the valence angle, OCO, having the value 125.40°; the three bond lengths, N-H, have the value 1.03 Å and the corresponding valence angles C-N-H the value 111.40°; these values, derived from a crystallographic study of the glycine zwitterion [28] and already adopted in a theoretical study of this molecule [29], seemed preferable to those of Ref. 27 in which the carboxylate group is not ionized. In addition, the valence angle, $C_2C_1N_5$, is taken to be equal to 108.6° instead of 107.3° in [27] and 111.9° in [29] and the valence angle, $C_1C_2H_4$, has the value of 108.1°.

The torsional angles are defined according to Sundaralingham's notations and convention [30]. Let us recall that (Fig. 2) for a bond, j, a torsion angle of 0° defines the planar-*cis* configuration of bonds j + 1 and j — 1, and a torsion angle of 180° defines their planar-*trans* configuration. The torsion angle is considered positive for a clockwise rotation to bring bond j — 1 (the closest one on the figure) superimposed over bond j + 1. The torsion angles of the serine phosphate backbone are defined as follows: $\alpha_2 = HO_{11}\text{-}P_8O_7$; $\alpha_3 = O_{11}P_8\text{-}O_7O_6$; $\alpha_4 = P_8O_7\text{-}C_6C_4$; $\alpha_5 = O_7C_6\text{-}C_4N_5$; $\alpha_6 = C_6C_4\text{-}N_5H$ and $\alpha_7 = C_6C_4\text{-}C_2O_1$.

We have selected eleven conformations of serine phosphate in such a way so that they permit a chelation of its anionic groups by the metal cation. This was done by imposing realistic interatomic distances between its atoms and distances between Ca^{2+} and the anionic oxygens involved in the complex close enough to, although consistently larger than, the known equilibrium distances,

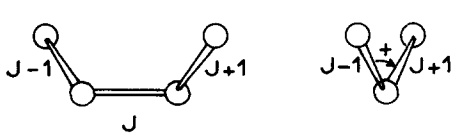

Fig. 2. Definition of torsion angles: sense of rotation and choice of zero angle.

Ca^{2+}-O, in the interaction of the cation with PO_4^- or COO^- in a standard B or E site. This study was performed on the D-isomer of serine phosphate. Identical results would be obtained with the L-isomer by inverting the signs of the torsional angles. Throughout this study, the value of α_2 (defining the orientation of the terminal hydrogen) was kept equal to −81°, which was also the value adopted in a theoretical study on ethanolamine phosphate [20], whereas the values of α_6 and α_7 (defining the conformation of the glycinium fragment) were kept equal to 180° and 240°, respectively, which yield the minimum-energy conformation of the glycine zwitterion [29]. One of the N-H bonds is therefore kept in a *trans* configuration with respect to the C-C bond of the ethanolamine phosphate fragment, whereas one of the C-O bonds of the glycinium fragment is kept in a *cis* configuration with respect to the C-N bond of this fragment.

A value of 240° for α_7 results in the torsion angles, $NC_3C_2O_1$ and $NC_3C_2O_3$, having the values 180° and 0°, respectively, which correlates with the experimental values of 178.7° and −2.6° for these angles [27]. This conformation of the glycinium group also corresponds to its theoretically computed minimum-energy conformation [29] and it may be noted that the rotation barrier around the C_2-C_4 bond is a high one: 6 kcal/mol for a 30° rotation around this bond and up to 14 kcal/mol for a 90° rotation.

Results

The complex SP-Ca^{2+}

The optimal interaction modes of SP-Ca^{2+} were investigated for the eleven conformations by varying the position of the cation through the use of a minimization program [31]. The total energy of a given SP-Ca^{2+} complex is the sum of the intramolecular energy of the isolated ligand and of the computed intermolecular interaction energy.

The results are reported in Table II, in which are listed for each conformer:

TABLE II

Interaction of Ca^{2+} with one serine phosphate molecule. The conformers are ordered following the decreasing values of α_5 (energies in kcal/mol). δE_0 is the intramolecular energy difference with respect to the energy of the most stable of the investigated eleven conformers. $\Delta E_{SP\text{-}Ca^{2+}}$ is the optimized interaction energy between serine phosphate and Ca^{2+}. δE is the total energy difference with respect to the most stable complex SP-Ca^{2+}. Torsion angles α_5, α_4 and α_3 are expressed as degrees.

Conformer	α_5	α_4	α_3	δE_0	$\Delta E_{SP\text{-}Ca^{2+}}$	δE
I	−23.5	−74.5	−146.5	0.0	−328.2	1.3
II	−25	−95	−65	10.4	−323.7	16.3
III	−30	−110	−60	9.6	−327.5	11.7
IV	−33.5	−89.5	−131.5	12.3	−340.5	1.4
V	−33.5	−110	−90	10.8	−330.7	9.7
VI	−34.8	−94.5	−161.5	19.8	−337.5	11.9
VII	−39.8	−89.5	−142.5	19.8	−348.8	0.5
VIII	−45.6	−104.5	−120.0	18.3	−347.8	0.0
IX	−63.5	−115	−90	28.1	−350.2	7.4
X	−96	−115	−90	43.0	−354.1	18.4
XI	−105.5	−142	−91	34.4	−339.8	24.2

the values of the torsional angles, α_5, α_4 and α_3; the intramolecular energy difference (δE_0) with respect to the energy of the most stable of these eleven conformers; the optimized interaction energy, $\Delta E_{SP\text{-}Ca^{2+}}$; and the resulting total energy difference with respect to the most stable complex SP-Ca^{2+}(δE). More detailed data pertinent to this and the following sections are available from the author upon request.

The most stable conformation of the complex is seen to be conformation VIII, in which Ca^{2+} interacts simultaneously with the four anionic oxygens of the ligand. However, conformers I, IV and VII, are seen to be energetically close to VIII. The proposed preferred arrangement is shown in Fig. 3. Figs. 3—5 have been drawn with the help of FIGATOM program (Fortran IV) for drawing stereoscopic views with a graphic plotter [32].

The complex SP-Ca^{2+}-SP

In this investigation of the modes of intermolecular chelation of two serine phosphates by Ca^{2+}, the carboxylate groups of the two ligands are constrained to be coplanar, and the corresponding C_4-N_5 bonds are *trans* with respect to

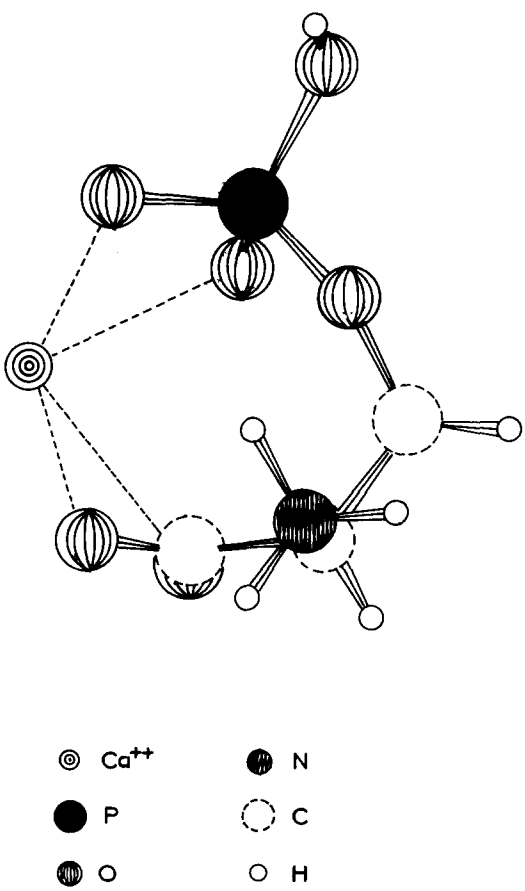

Fig. 3. The intrinsically preferred mode of interaction of Ca^{2+} with one serine phosphate molecule.

each other, so that Ca^{2+} be interposed between its two ligands.

In a first investigation, the two serine phosphates are fixed in the same conformation and a search is performed for their most stable mutual arrangement, together with an optimization of the position of the cation. This investigation was restricted to conformers I, IV, VIII, IX and X. α_5 and α_4 decrease from $-23.5°$ to $-96°$ and from $-74.5°$ to $-115°$, respectively, whereas α_3 increases from $-146.5°$ to $-90°$.

The total energy of the supersystem, SP-Ca^{2+}-SP, is the sum of twice the intramolecular energy of serine phosphate in the corresponding conformation and of the intermolecular interaction energy $\Delta E_{SP\text{-}Ca^{2+}\text{-}SP}$ (the interaction energy between the two ligands is included in this term). Reported in Table III are the values of $\Delta E_{SP\text{-}Ca^{2+}\text{-}SP}$ for the respective arrangements and the total energy difference, δE_T, with respect to the most stable one.

It is seen that the intermolecular interaction energy increases (in absolute values) from the arrangement I-I to X-X but its contribution is opposed by the reverse evolution of the intramolecular energy of the conformers which is now multiplied by two. The balance of these two trends favours the involvement of conformer I in the association studied. It is then concluded that the interaction of Ca^{2+} with two serine phosphate molecules, constrained to adopt the same conformation, intrinsically prefers conformer I. In this conformation, one anionic oxygen of the phosphate of each ligand (O_9) is in the vicinity of one hydrogen of the corresponding ammonium group, the remaining anionic oxygens interacting with Ca^{2+}. This result is similar to that of Ref. 17 in which it was found that the interaction of Na^+ with one serine phosphate molecule displaces it from its intrinsically preferred conformation and stabilizes preferentially the same type of conformation. Computations in which the two ligands adopt different conformations have also been done in a limited number of cases

TABLE III

Investigation of the intrinsically preferred chelation modes of two serine phosphate molecules (constrained to adopt the same conformation) by Ca^{2+}. $\Delta E_{SP\text{-}Ca^{2+}\text{-}SP}$ is the total intermolecular interaction energy in the complex SP-Ca^{2+}-SP. δE_T is the total energy difference with respect to the most stable investigated arrangement.

Conformers	$\Delta E_{SP\text{-}Ca^{2+}\text{-}SP}$ kcal/mol	δE_T kcal/mol
I-I [a]	−506.9	0.0
IV-IV [b]	−523.7	7.9
VIII-VIII [a]	−528.1 [d]	15.5
VIII-VIII [b]	−529.7 [e]	13.9
IX-IX [b]	−535.1	28.0
X-X [c]	−541.7	51.3

[a] The segments $O_1'O_3$ and $O_3'O_1$ are equal and parallel (the atoms of the second serine phosphate are denoted by primes).

[b] O_1' is located on the first bisector of the angle $O_1C_2O_3$ and the distances $O_1'O_1$ and $O_3'O_1$ are equal.

[c] The values of angles $O_1'O_1C_2$ and $C_2'O_1'O_1$ are 180° and 146°, respectively.

[d] The optimized position of the cation is in the vicinity of the eight anionic oxygens (intermolecular distances, $d_{Ca^{2+}\text{-}O}$, in the range 2.55—2.70 Å).

[e] The optimized position of the cation is closer to $O_1,O_{10},O_1'O_{10}'$ (average distance, $d_{Ca^{2+}\text{-}O}$ = 2.45 Å) and farther from O_3,O_9,O_3',O_9' (average distance $d_{Ca^{2+}\text{-}O}$ = 2.90 Å).

and were found to be consistently less stable than the arrangement I-I (unpublished data).

The complex, SP-Ca^{2+}-SP, in the arrangement denoted I-I is shown in Fig. 4.

In a second investigation, an alternative model for the construction of the chelates, SP-Ca^{2+}-SP, was examined. The aim of this study was to test a plausible assumption, according to which the complex PS-Ca^{2+}-PS consists of repetitive chelates, deduced by successive translations, such that either the COO^- or the PO_4^- group of every second phosphatidylserine molecule interacts with the ammonium group of the first phosphatidylserine molecule of the neighboring chelate.

For this purpose we have investigated chelates in which the first serine phosphate molecule adopts one of the five conformations of Table III, whereas the second consistently adopts a conformation defined by the set of angles: $\alpha_2 = -81°$, $\alpha_3 = -88.6°$, $\alpha_4 = -90°$ and $\alpha_5 = +90°$ (denoted as conformer A). In this

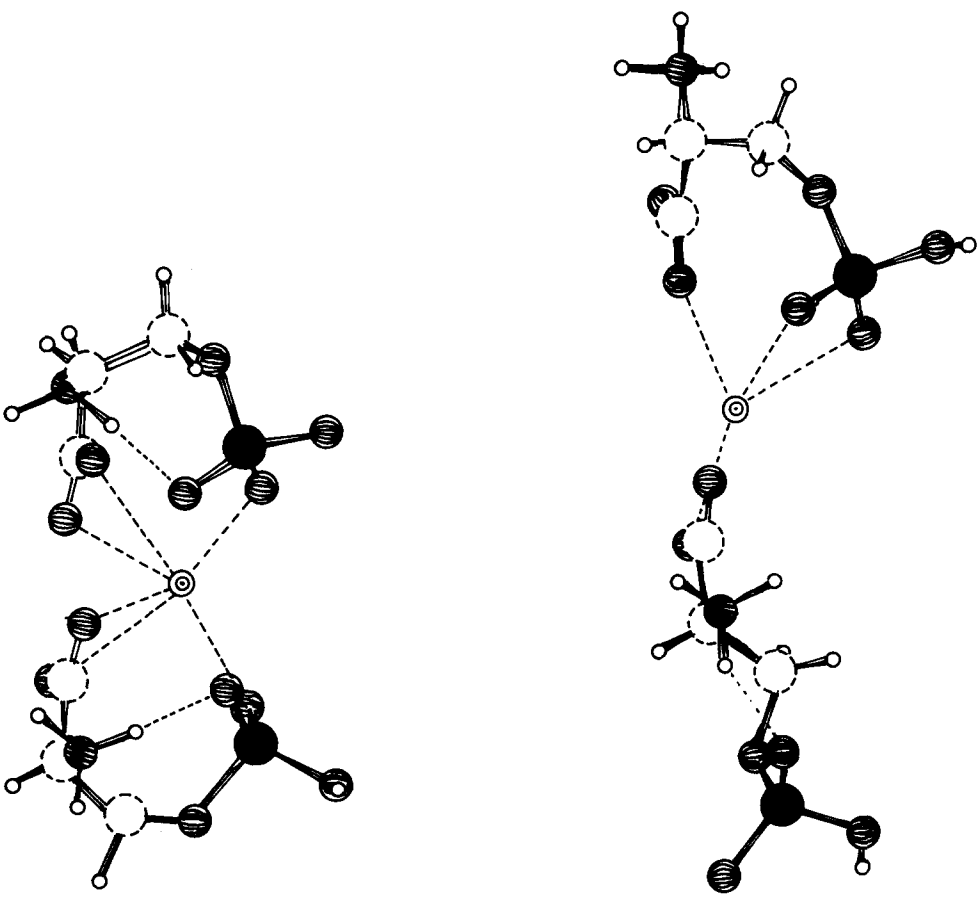

Fig. 4. The intrinsically preferred mode of chelation of two serine phosphate molecules by Ca^{2+}. The symbols used are the same as those defined in Fig. 3.

Fig. 5. The chelate, SP-Ca^{2+}-SP, in arrangement X-A. Ca^{2+} bound to the carboxylate group of conformer A. The symbols used are the same as those defined in Fig. 3.

conformation, the phosphate and the carboxylate groups are kept apart from each other and an intramolecular hydrogen bond takes place between one anionic oxygen of the phosphate and one hydrogen of the ammonium group ($d_{O_{10}\text{-}H}$ = 1.68 Å). This conformation was selected because it is in the vicinity of the global minimum of isolated serine phosphate [17]. Other possibilities for conformer A cannot be precluded, provided that the phosphate and the carboxylate groups are kept apart from each other.

Conformer A was bound through its carboxylate group to Ca^{2+}, so that its phosphate group could interact electrostatically (through O_9) with the ammonium group of the first ligand in the neighboring chelate. An alternative possibility, also investigated, is one in which the second ligand is bound to Ca^{2+} through its phosphate group, so that it is the carboxylate group that would interact with the ammonium group of the neighboring chelate.

For Ca^{2+} interacting with conformer A through its carboxylate group, the optimized energetical ordering of the chelates is (in decreasing stabilities): I-A > X-A > IV-A > IX-A > VIII-A, the four arrangements being less stable than I-A by 1.3, 1.9, 3.8 and 5.2 kcal/mol, respectively. For Ca^{2+} interacting with conformer A through its phosphate group the ordering is: I-A > IX-A > X-A. The interaction of Ca^{2+} with conformer A through its carboxylate group is preferred to that through its phosphate group by 8.5 and 14.8 kcal/mol in arrangements I-A and X-A, respectively. In marked contrast with the results of Table III (in which chelate X-X was less stable than chelate I-I by 51 kcal/mol), all investigated combinations of conformers, from I-A to X-A, are seen to be of very close overall stabilities, with Ca^{2+} interacting with conformer A through its carboxylate group. Chelate X-A (shown in Fig. 5) is, in particular, only 1 kcal/mol less stable than chelate I-A. This arrangement remains, however, 48 kcal/mol less stable than arrangement I-I.

The complexes SP-Mg^{2+} and SP-Mg^{2+}-SP

The interactions, SP-Mg^{2+}, were investigated in the same way as the interactions, SP-Ca^{2+}: this search was restricted, however, to conformers I, IV, VIII, IX and X. The optimized interatomic distances, Mg^{2+}-$O^{\delta-}$, are in the range expected for a 3- or a 4-fold coordination of this cation (unpublished data). The only exception is conformation IX, in which Mg^{2+} is in a much closer vicinity to O_1 and O_{10} (average distance, 1.85 Å) than to O_3 and O_9 (average distance, 2.84 Å). The interaction energies have the following values (in kcal/mol): −383.2, −394.7, −399.4, −404.1 and −418.3, respectively.

The most stable conformation of the complex is conformation I, although conformation VIII is only 2 kcal/mol less stable.

The interactions, SP-Mg^{2+}-SP, were investigated in a manner similar to the interactions, SP-Ca^{2+}-SP, on the following arrangements: I-I, VIII-VIII, X-X, I-A, VIII and X-A. The results are reported in Table IV which lists the intermolecular interaction energy, $\Delta E_{SP\text{-}Mg^{2+}\text{-}SP}$, the value of the difference, $\delta = \Delta E_{SP\text{-}Ca^{2+}\text{-}SP} - \Delta E_{SP\text{-}Mg^{2+}\text{-}SP}$, the first term being the one computed in the preceding section for the same combination of conformers, and the total energy difference with respect to the most stable arrangement (δ_1).

Arrangement I-I is seen to be the most stable one, as was the case with Ca^{2+}. Interestingly, arrangement X-A is the most stable of the investigated arrange-

TABLE IV

Investigation of the interaction modes SP-Mg^{2+}-SP (energies in kcal/mol). (a) The segments O'_1O_3 and O'_3O_1 are equal and parallel. (b) O'_1 is located on the first bisector of angle $O_1C_2O_3$ and the distances O'_1O_1 and O'_3O_1 are equal. (c) Binding of conformer A to Mg^{2+} through its carboxylate group. (d) Binding of conformer A to Mg^{2+} through its phosphate group. $\Delta E_{SP\text{-}Mg^{2+}\text{-}SP}$ is the intermolecular interaction energy in the complex SP-Mg^{2+}-SP. δ is the value of the difference: $\Delta E_{SP\text{-}Ca^{2+}\text{-}SP} - \Delta E_{SP\text{-}Mg^{2+}\text{-}SP}$, the first term being the one computed in the preceding section for the same combination of conformers. δ_1 is the total energy difference with respect to the most stable arrangement of the complex SP-Mg^{2+}-SP.

Conformers	$\Delta E_{SP\text{-}Mg^{2+}\text{-}SP}$	δ	δ_1
I-I (a)	—554.6	47.7	0.0
VIII-VIII			
(a)	—566.6 *	38.5	24.6
(b)	—577.7 **	48.0	13.5
X-X	—607.0	65.2	33.6
I-A (c)	—478.7	47.1	47.3
VIII-A (c)	—493.7	49.1	50.6
X-A			
(c)	—547.0	73.7	22.0
(d)	—529.9	71.4	39.1

* The optimized position of the cation is in the vicinity of the eight anionic oxygens (intermolecular distances, $d_{Mg^{2+}\text{-}O}$ in the range 2.35—2.55 Å).

** The optimized position of the cation is closer to $O_1, O_{10}, O'_1O'_{10}$ (average distance, $d_{Mg^{2+}\text{-}O}$ = 2.20 Å) and farther from O_3, O_9, O'_3, O'_9 (average distance, $d_{Mg^{2+}\text{-}O}$ = 2.70 Å).

ments involving conformer A. With Mg^{2+} interacting with conformer A through its carboxylate group, X-A is more stable than I-A by 25 kcal/mol, whereas with Ca^{2+}, I-A and X-A were of comparable stabilities.

Discussion

Upon investigating the various interaction modes, SP-M^{2+} and SP-M^{2+}-SP, it is seen that the computed intermolecular interaction energies are consistently larger for Mg^{2+} than for Ca^{2+}, owing to the smaller ionic radius of the former, allowing it a closer approach to its ligand(s). A comparison between the overall binding energetics of Mg^{2+} and Ca^{2+} requires, however, taking also into account their respective dehydration energies and assessing their coordination numbers.

The experimental hydration enthalpies of Mg^{2+} and Ca^{2+} are —459 and —377 kcal/mol respectively [33], so that there is a difference of 82 kcal/mol in favour of Mg^{2+}. X-ray diffraction results show that the coordination number of Mg^{2+} is generally six [34—36], whereas that of Ca^{2+} may vary from six to eight [37—39].

A comparison between the binding energetics of Mg^{2+} and Ca^{2+} is therefore possible when considering the arrangements I-I to X-X. These correspond to extreme cases in which either cation is completely dehydrated and maximally coordinated with anionic oxygens. This assumption is certainly justified in the case of (PS-Ca^{2+})complex which was demonstrated experimentally to have no tightly-bound water [9]. Formation of chelates, SP-M^{2+}-SP, in these arrangements occurs more favourably with Mg^{2+} than with Ca^{2+} by amounts which are

unsufficient to compensate for the difference in the dehydration energies of Mg^{2+} and Ca^{2+}, namely 82 kcal/mol. The overall energy balance is therefore in favour of Ca^{2+}, and the displayed selectivity is larger for arrangement I-I than for arrangement X-X, inasmuch as the difference in the interaction energies, $\Delta E_{SP\text{-}M^{2+}\text{-}SP}$, is of 48 kcal/mol in favour of Mg^{2+} in arrangement I-I, and increases up to 65 kcal/mol in arrangement X-X. In the special case when both cations have an 8-fold coordination, as in VIII-VIII (a), this difference is reduced to 38.5 kcal/mol.

When considering arrangement X-A, it is seen that the interaction energy, SP-M^{2+}-SP, favours Mg^{2+} over Ca^{2+} by 74 kcal/mol, which is much closer to the difference in the dehydration energies of these cations. Moreover, these cations are not maximally coordinated and the spacing of the two serine phosphate groups in arrangement X-A should allow for additional interactions with approaching water molecule(s), in which case arrangement X-A could ultimately favour Mg^{2+} over Ca^{2+}. On the other hand, it is noteworthy that with Mg^{2+} interacting with conformer A through its carboxylate group, arrangement X-A is only 22 kcal/mol less stable than arrangement I-I. With Ca^{2+} interacting, this difference amounted to 48 kcal/mol in favour of I-I.

The present calculations assume definite conditions with respect to the ionization state of the ligand(s) and the stoichiometry of the envisaged complexes: their scope is specific to the two alkaline-earth cations, Ca^{2+} and Mg^{2+}. It should then be noted that only experimental situations, dealt with when these assumptions are fulfilled can be compared to the present results, the generalization of which, under differing experimental conditions, has to be investigated.

The essential results of this work can be summarized in the following way:

(a) Upon going from arrangement I-I to arrangement X-X:

The intramolecular (conformational) energy of serine phosphate decreases:

The intermolecular interaction energy of SP-M^{2+}-SP increases, the overall balance of these two trends decreasing so that arrangement I-I is the preferred one with either Ca^{2+} or Mg^{2+};

The displayed selectivity for Ca^{2+} becomes less pronounced.

(b) Mg^{2+} distinctly stabilizes arrangement X-A over I-A or VIII-A, in contrast to Ca^{2+}, and with Mg^{2+} interacting with conformer A through its carboxylate group, the overall energy of this arrangement comes closer to that of arrangement I-I.

The investigation carried out on arrangements I-I to X-X provides a basis for possible models for the complex formed by Ca^{2+} and two phosphatidylserine molecules in model membranes:

(a) It indicates the trend in energy for the conformation of serine phosphate as defined by the torsional angles α_3, α_4 and α_5. Arrangements such as I-I or VIII-VIII should be the most likely ones, so that: $-150° < \alpha_3 < -120°$; $-105° < \alpha_4 < -70°$; $-50° < \alpha_5 < -23.5°$. For the L-isomer of phosphatidylserine, $150° > \alpha_3 > 120°$; $105° > \alpha_4 > 70°$; $50° > \alpha_5 > 23.5°$.

(b) It accounts for the selectivity of the binding of phosphatidylserine to Ca^{2+} rather than to Mg^{2+}, the difference in dehydration energies of Mg^{2+} and Ca^{2+} being much larger than the differences in the interaction energies $\Delta E_{SP\text{-}Mg^{2+}\text{-}SP}$ and $\Delta E_{SP\text{-}Ca^{2+}\text{-}SP}$.

The polymer $(PS\text{-}Ca^{2+}\text{-}PS)_n$ in model membranes could then consist of two rows of serine phosphate facing each other in configurations similar to the ones investigated in this work, so that two consecutive serine phosphate molecules in each row can be translationally related. The intermolecular linking of two chelates should, however, require a different coordination of the cation than in the isolated dimers. Two chelates such as VIII-VIII would be linked by Ca^{2+} chelating four serine phosphate molecules through two anionic oxygens from every chelate (namely, O_1 O_{10}, O_3' O_9', O_3'' O_9'' and O_1''' O_{10}''').

Electrostatic interactions involving the $-NH_3^+$ group and the anionic oxygens of a neighbouring serine phosphate group (O_3 and O_9) may occur in a concurrent manner. A more thorough approach would require explicit computations on arrangements such as $(SP\text{-}Ca^{2+}\text{-}SP)_2$; such computations, although still tractable in the framework of the empirical procedure, are beyond the scope of this work.

In this connection, it is noted that the model suggested for the juxtaposition of chelates VIII-VIII bears a strong resemblance to the model proposed by Papahadjopoulos in 1968 for the complex $(PS\text{-}Ca^{2+}\text{-}PS)_n$ [3]. This model indeed consists of a linear polymeric arrangement involving coordination bonds between one Ca^{2+} and four phosphatidylserine molecules, whereas Ca^{2+} is assumed to be 6-fold coordinated.

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