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Cation Binding to Biomolecules

V. Binding of Alkali and Alkaline-Earth Cations to the Phosphate Group

Conformational Effects on the Phosphodiester Linkage and the Polar Head of Phospholipids

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SCF *ab initio* computations are carried out on the binding of alkali and alkaline-earth cations to the phosphate monoanion. The effect of the binding on the conformational properties of the phosphodiester linkage and of the polar head of phospholipids is investigated. The results indicate that following the nature of the cation and the site of its binding, the interaction may have a profound influence on the conformation of the ligand. The consequences of this situation on the use of lanthanide probes in NMR studies are considered.

Key words: Biomolecules, cation binding to ~ - Phosphate group, binding of alkali and alkaline-earth cations to ~

As a continuation of our studies on the effect of environmental factors on the structure and properties of fundamental biomolecules [1–4], we have investigated the cation-binding properties of the phosphate monoanion which represents an essential constituent of the nucleic acids and of the phospholipid components of membranes. We have extended the study to the evaluation of the effect of the cation binding on the conformational properties of the phosphodiester linkage and of the polar head of phospholipids. This work thus continues our previous studies on the conformational preferences of these same systems in free space [5, 6] and in water surroundings [7, 8].

1. Computational Details

As in Refs. [5] and [7] we have used the dimethylphosphate anion (DMP^-, Fig. 1) as a model compound for the phosphodiester linkage. In a previous note [9] preliminary results have been described on the interaction between Na^+ and Mg^{++} cations and DMP^- as obtained by the SCF *ab initio* procedure using the STO 3G basis set [10]. The work has presently been refined by using a more elaborate basis set and extending the computations to K^+ and Ca^{++} besides Na^+ and Mg^{++}.

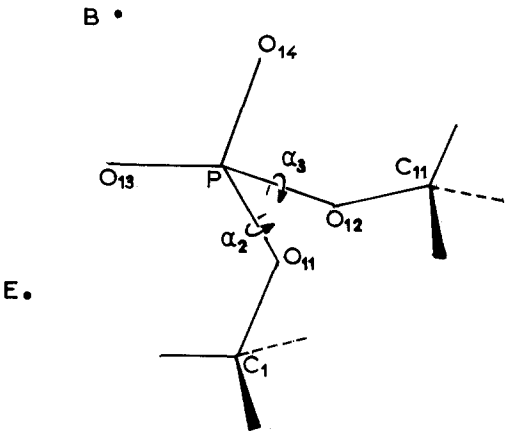

Fig. 1. The dimethylphosphate anion; atom numbering, torsion angles and preferred sites for cation binding

The incentive for exploring more elaborate basis sets sprung from the observation that the use of the standard STO 3G basis for studies of cation binding results in too strong interaction energies and too short interaction distances [11]. We have analysed elsewhere [12] the reasons for these defects and the possibilities of avoiding them while remaining inside the limits of computational feasibility for large ligands. This study has shown that the use of a standard STO 3G basis on both the ligand and the cation gives rise to an exceptionally large spurious stabilization of the cation-ligand super-system with respect to the sum of the energies of the individual components, due to the particularly strong unbalance of the basis set created by the presence on the cation of the twelve Gaussian functions of the empty valence shell. Upon the approach of the ligand, itself poorly represented in an STO 3G basis, the electrons flow into the easily accessible empty orbitals of the cation, artificially lowering the energy through its charge-transfer component. We have suggested a convenient way to remedy this situation, namely to suppress a part of the empty valence orbitals on the cation (while reoptimizing the exponents of the inner shells) and simultaneously improving as much as possible, within the limits of a minimal basis, the intrinsic representation of the ligand. A detailed investigation of the hydration energies of alkali and alkaline-earth cations has shown [12] that reasonable values could be obtained using the Gaussian basis given in Table 1 for the cations, associated with those of Table 2 for the first-row atoms. These bases are those adopted in the present work.

The Gaussian set utilized on the phosphorus atom given in Table 2 has been chosen as follows: the exponents are those of an atom-optimized (10*s*, 6*p*) set [13] contracted to minimal as indicated and supplemented by six *d* Gaussian functions so as to correctly

Table 1. Reoptimized STO 3G basis for the cations [12]

	1*s*	2*sp*	3*sp*	4*sp*
Na^+	10.68	3.51	1.95[a]	
K^+	18.60	7.26	2.72	2.12[a]
Mg^{++}	11.68	3.99	2.33[a]	
Ca^{++}	19.60	7.74	3.02	2.66[a]

[a] *p* orbitals suppressed in the valence shell

represent the pentavalent phosphorus of DMP^- [14]. The contraction has been chosen so as to be coherent with that adopted for the first-row atoms, while keeping a minimal loss of accuracy in energy. The remaining loss in accuracy by contraction is further corrected by the adjunction of the *d* functions.

As an illustration of the accuracy of the basis sets utilized, we give below a few typical results concerning the dimethylphosphate anion itself. Thus Table 3 gives a comparison of the values of the total energy for DMP^- in its *gauche-gauche* conformation computed

Table 2. Gaussian exponents and contraction coefficients utilized on the P, C, O, H atoms of DMP^-

Atom	Type	Exponent	Coefficient
P	*s*	22566.5	0.0015310
	s	3380.80	0.011793
	s	766.417	0.058861
	s	214.964	0.208183
	s	68.9703	0.447369
	s	23.9195	0.390968
	s	5.26929	0.441137
	s	1.96297	0.667165
	s	0.350435	0.557322
	s	0.131021	0.609385
	p	109.959	0.028840
	p	25.1292	0.175866
	p	7.51127	0.461765
	p	2.39583	0.490526
	p	0.531089	0.391025
	p	0.150160	0.729140
	d	0.380000	1.0
O	*s*	1113.12	0.013221
	s	172.26	0.087629
	s	42.8008	0.296295
	s	13.3710	0.492042
	s	4.8397	0.258935
	s	1.0738	0.497086
	s	0.3169	0.566094
	p	6.922	0.148880
	p	1.4261	0.516709
	p	0.3212	0.558700
C	*s*	391.445	0.02222
	s	64.7358	0.132968
	s	16.2247	0.384690
	s	5.3346	0.458385
	s	2.00995	0.154547
	s	0.502323	0.534240
	s	0.155139	0.524992
	p	4.31613	0.108451
	p	0.873682	0.461166
	p	0.202860	0.630436
H	*s*	6.48053	0.070480
	s	0.981039	0.407890
	s	0.217979	0.647669

Table 3. Energy characteristics of DMP^- (*gg*) in various basis sets

Basis	a	b	c	d
Total energy (a.u.)	−710.21747	−710.69283	−717.08732	−717.51230
Homo (a.u.)	+0.04855	+0.02440	−0.10804	−0.13330
n^e	3	1	0	0

[a] STO 3G [7].
[b] STO 3G + *d* [14].
[c] Present work without *d*.
[d] Same with *d* (see text).
[e] *n* is the number of occupied molecular orbitals with positive energy.

with the basis set of Table 2 with and without *d* functions, compared to the corresponding values obtained in our former STO 3G and STO 3G + *d* computations [7, 14]. The improvement of the minimal basis by the adjunction of the *d* functions is similar in the two cases, but the total energy is appreciably lowered here with respect to the STO 3G and the STO 3G + *d* values respectively.

The location of the highest filled molecular orbital in the energy scale is another test for assessing the more or less satisfactory character of an SCF computation for an anion [15]: according to this criterion, only when the *homo* is bonding is the solution really satisfactory. It is seen in Table 3 that this criterion is satisfied with the basis set of Table 2, even without the addition of the *d* functions. This is not the case when the standard STO 3G basis is used; in that case, although the improvement by addition of the *d* functions was in the proper direction, there was still a slight antibonding character in the *homo*.

A final illustration of the effect of the *d* functions on the results is given in Fig. 2 which shows the net charges in DMP^- obtained in a Mulliken population analysis without and

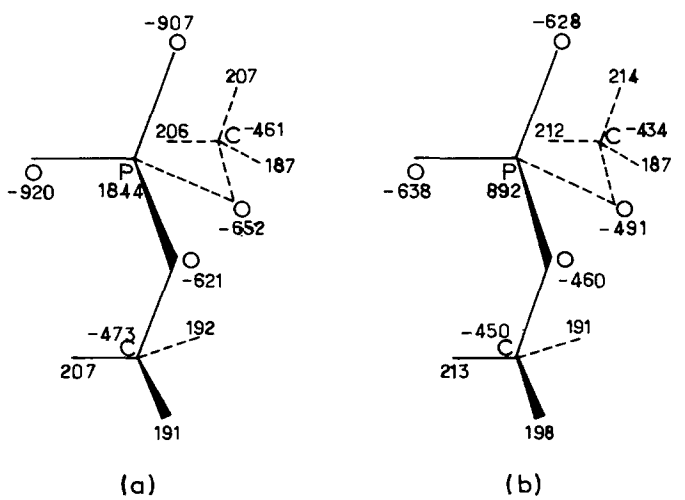

Fig. 2. Net atomic charges in DMP^- (*gt* conformer (300°, 180°)) a) without *d* functions on the phosphorus atom, b) with *d* functions on the phosphorus atom

with the inclusion of the *d* functions. The results may be compared to the corresponding data of Ref. [14]. Although the numerical values are different, the qualitative conclusions are the same as before and we do not repeat them.

2. Binding of Cations and the Conformation of DMP^-

Because of the high cost of *ab initio* computations, the investigation was limited to the fixation of the metal cations in the two most plausible sites, as indicated by the previous STO 3G computations [9]. Using the notations of Ref. [9], inspired from the notations used for the water fixation scheme [7], these sites are (Fig. 1): *B* on the bisectrix of the O_{13}-P-O_{14} angle (designated as B_{13} in Ref. [9]) and *E* along the direction making an angle of 120° with the $O_{13}P$ axis on the external site of the O_{13}-P-O_{14} angle (designated as E_{13} in Ref. [9]). For the same reasons of economy, the exploration of the conformational consequences of cation binding was limited to the evaluation of the effect of such attachments upon the relative energies of the three fundamental types of conformation resulting from possible rotations about the P-O_{ester} bonds: *gauche-gauche* (*gg*), *gauche-trans* (*gt*) or *trans-gauche* (*tg*) and *trans-trans* (*tt*) [5].

The principal results relevant to the energies of binding and the influence of binding upon the stability of the conformers are summed up in Table 4. The main conclusions which can be drawn from this study seem to be the following:

1) First, from the technical point of view, it may be observed that the energy differences between the conformers of free DMP^- are somewhat smaller when computed with the present basis set than when obtained in the STO 3G computations. The same general picture is nevertheless maintained: the *gg* conformation is the most stable one, followed by the *tg* (or *gt*, they are equivalent) 2.3 kcal/mole above it, followed in turn by the *tt*, 5.7 kcal/mole less stable than the *gg* one. From the same point of view it may be observed that, as expected [12], the energies of cation binding have been appreciably reduced and the equilibrium distances increased with respect to the results of the STO 3G calculation (compare with Table 1 in Ref. [9]). The reduction factor in the binding energies is 0.7, the same as that found for the interaction energies of the same cations with water [12] computed with the corresponding basis sets. The strongest binding continues, however, to correspond to the *B* site. As expected, Na^+ and Mg^{++} bind more strongly than K^+ and Ca^{++}, respectively, the two divalent cations binding more strongly than the monovalent ones. The order of the binding energy is $Mg^{++} > Ca^{++} > Na^+ > K^+$, which is the intrinsic order of binding of these cations to a given well-defined site [12].

2) The fixation of the cations at the *B* site does not influence the order of conformational preferences with respect to the torsion about the P-O_{ester} bonds existing in the free DMP^-: the *gg* conformation remains the most stable, followed by the *gt* (*tg*) one, followed in turn by the *tt* one. In fact, as was visible already in the STO 3G computation, the cation binding in its preferred *B* site increases even the relative stability of the *gg* form with respect to the two others: it is now 4.3 kcal/mole more stable than the *gt* (or *tg*) one and 10 kcal/mole more stable than the *tt* one when the bound cations are the monovalent Na^+ and K^+; it is about 7 kcal/mole more stable than the *gt* (*tg*) one and about

Table 4. Cation binding to DMP^-

Site of Cation Binding	The Cation	$d_{O \ldots M^+}$	Energy of Cation Binding: ΔE with Respect to DMP^- and Cation at Infinite Separation				Difference in Energy of Cation Binding			Difference in Energy between Conformers with Bound Cation		
			gg	*gt*	*tg*	*tt*	ΔE (*gt-gg*)	ΔE (*tg-gg*)	ΔE (*tt-gg*)	ΔE (*gt-gg*)	ΔE (*tg-gg*)	ΔE (*tt-gg*)
Free DMP^-										2.3	2.3	5.7
B	Na^+	2.15	−150.3	−148.3	−148.3	−146.0	2.0	2.0	4.3	4.3	4.3	10.0
	Mg^{++}	1.95	−340.1	−335.2	−335.2	−330.0	4.9	4.9	10.1	7.2	7.2	15.9
	K^+	2.5	−128.3	−126.3	−126.3	−124.1	2.0	2.0	4.2	4.3	4.3	9.9
	Ca^{++}	2.3	−285.0	−280.3	−280.3	−275.2	4.7	4.7	9.8	7.0	7.0	15.4
E	Na^+	2.0	−118.6	−115.8	−125.5	−122.7	2.8	−6.9	−4.1	5.1	−4.7	1.6
	Ca^{++}	2.15	−227.0	−220.3	−240.0	−233.5	6.7	−13.0	−6.5	8.5	−10.8	−1.3

15 kcal/mole more stable than the *tt* one when the bound cations are the divalent Mg^{++} and Ca^{++}.
3) On the contrary, the binding of the cations to the external *E* site is able to produce modifications in the previous order of conformational stabilities, the perturbations being stronger with the divalent Ca^{++} than with the monovalent Na^{+}. With the monovalent Na^{+} the *tg* form becomes the most stable one, followed, in decreasing order of stability, by *gg*, *tt* and *gt*; with the divalent Ca^{++} both the *tg* and the *tt* conformers become more stable than the *gg*, only the *gt* being less stable.

The essential conclusion which can be drawn from these results is that, according to the site of binding, the cations may or may not perturb the intrinsic conformational preferences of DMP^{-}. There are no direct experimental results which may be compared with the theoretical predictions. X-ray crystallographic results on related systems show a conservation of the *gg* conformation in some (barium diethylphosphate [16], magnesium diethylphosphate [17] and glycerylphosphorylcholine $CdCl_2$ trihydrate [18]) but not in others (silver diethylphosphate [19]). The crystal data generally correspond, however, to complex interactions, involving a number of entities, and cannot thus be easily compared with the theoretical ones, which must at present be considered as primarily model studies on essential possibilities. As such, however, they may be quite illustrative. Thus the pre-eminence of the tg^{-} conformation in the presence of cations at the *E* site may be of particular significance in connection with Sundaralingam's model for the configuration of the *t*RNA-*m*RNA complex on the ribosome during peptide bond synthesis [20]. The stereochemical requirements for the interaction of messenger RNA with the aminoacyl and peptidyl transfer RNA's are best satisfied when the phosphodiester group bridging the adjacent codon triplets of *m*RNA is in the extended tg^{-} conformation, which could be produced by the binding of Mg^{2+}, the presence of which is known to be necessary for the process to occur.

The present results do not modify our previous estimation [9] of the inadequacy of the CNDO method for this kind of investigation, which was based on the disagreement between SCF *ab initio* and CNDO predictions as to the effect of cation binding at the *B* site of DMP^{-} upon its preferred conformation. The present, more refined computations, confirm previous *ab initio* results which were obtained with an STO 3G basis set.

The perturbation brought about by the presence of a bound cation on DMP^{-}may be illustrated further by its effect on the electronic distribution. Examples of these perturbations are given in Table 5 for three typical adducts compared to the isolated molecule: one monocation Na^{+} and one dication Ca^{++} at the same site *E* (at their respective equilibrium distances) and the dication Ca^{++} in the bridge site *B*. The site *E* involved is on the side of O_{14}. The conformer of DMP^{-} is the *gt* one in all cases. It is seen, as observed before (see Ref. [12]), that the charge transfer to the cation is small, the overall effect on the electron distribution being a strong polarization towards the atoms closest to the cation. This is noted by the fact that the negative charge on O_{14} increases by 0.081 for sodium bound to it and by 0.066 more for binding of the di-cation to the same site, the draining of the charge towards this atom occurring at the expense of the other positions. Similarly this polarizing effect (development of extra negative charge)

Table 5. Net charges (10^{-3} electron units) in various adducts of DMP^- (*gt*) with Na^+ and Ca^{++}

Cation Site	None -	Na^+ *E*	Ca^{++} *E*	Ca^{++} *B*
O_{13}	−638	−601	−569	−720
P	892	+896	+877	+893
O_{14}	−628	−709	−775	−708
O_{12}	−491	−489	−490	−454
O_{11}	−460	−479	−492	−450
C_{11}	−491	−439	−440	−438
C_1	−450	−441	−439	−441
H_{11}	187	207	225	246
H'_{11}	214	196	179	205
H''_{11}	212	234	256	202
H_1	198	206	219	235
H'_1	191	209	220	228
H''_1	213	234	249	219
Cation	-	975	1980	1982

may be observed on the hydrogen atom closest to the cation: whereas all the hydrogens become more positive by draining out of the electrons, the positive charge on H'_{11} becomes less positive upon binding of the cation to O_{14}, with a larger effect for calcium than for sodium. When the cation is in the bridge site both O_{13} and O_{14} undergo negative polarization.

3. Binding of Cations to the Phosphate Group and the Conformation of the Polar Head of Phospholipids

In this section we present the results of a preliminary exploration of the effect of cation binding to the phosphate group upon the conformation of the polar head of phospholipids.

As a model compound for this polar head, we have used (as in Refs. [6] and [8]) ethanolamine phosphate (EP, Fig. 3). In this compound the torsion angles α_4 and α_5 (in Sundaralingam's notation, Ref. [21]) are those which determine the overall conformation of the polar head [6-8]. One of the essential results of our previous SCF *ab initio* computations on the conformation of the polar head of phospholipids was to show that the intrinsically preferred conformation of this system corresponds

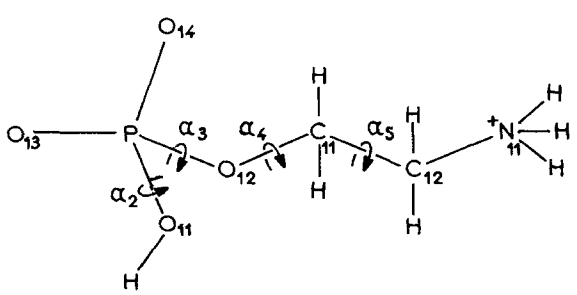

Fig. 3. Phosphatidylethanolamine: atom numbering and torsion angles

to a highly folded structure associated with $(\alpha_4, \alpha_5) = (270°, 30°)$. This structure results from strong intramolecular interactions between the cationic $-N^+=$ end group and the anionic phosphate group. NMR studies confirm the presence of such structures in non-polar solvents [22]. In water, the solvent should have the effect of extending the structure, in particular with respect to α_4 which, following the degree of hydration, tends towards 180° [8].

For the sake of homogeneity, the conformational energies of EP itself were first re-evaluated using the improved basis set described in Part 1. The exponents of the Gaussian functions for the hydrogen atoms of the ammonium end of the molecule are those optimized for the ammonium ion in Ref. [12]. These correspond to a Slater exponent $\zeta = 1.5$, while those of the ordinary hydrogens in Table 2 correspond to $\zeta = 1.2$. The bond lengths and bond angles adopted are the crystallographic data [23]. The torsion angles α_2 and α_3 were fixed at their crystallographic values $(-81°, -81°)$.

The results show great similarity to the previous ones [9], in particular the location of the global energy minimum at $(\alpha_4, \alpha_5) = (270°, 30°)$ remains practically unchanged.

The effect of cation binding upon this structure was studied for Na^+ and Ca^{++} attached at the *B* and *E* binding sites of the phosphate group, the cations being placed at the optimal distances found in the study of their interaction with DMP^- (Sect. 2). For a cation attached in position *B* the terminal OH hydrogen of EP was fixed in the *gauche* conformation about the phosphodiester bond (with $\alpha_2 = -81°$). In the case of a cation attached to position *E* (on the side of O_{13}), this hydrogen was fixed in a corresponding *trans* conformation ($\alpha_2 = 180°$) so as to take into account the conformational preferences found for DMP^- in the presence of a cation in the *B* and in the *E* positions, respectively (Sect. 2).

The results obtained with Na^+ are summed up in Tables 6 and 7 which give two kinds of information: ΔE_1 represents the interaction energies of the cation, fixed at sites *B* and *E*, with EP, as a function of α_4 and α_5, taken with respect to the energy of the most stable conformation of free EP. ΔE_2 represents the value of the energy of the adducts for a given value of (α_4, α_5), with respect to the most stable conformation of the adduct taken as energy zero. Because of the high cost of the computations only selective points of the α_4, α_5 map have been evaluated. They seem, however, to lead to clear-cut conclusions.

Thus it is seen that for the adduct at *B* a number of combinations of α_4-α_5 correspond to more favourable interaction energies ΔE_1 than the one associated with the minimum of the free EP $(\alpha_4, \alpha_5) = (270°, 30°)$. It is therefore not astonishing to observe on the ΔE_2 values that the most stable conformation of this adduct is substantially different from the most stable conformation of free EP. In particular, the preferred conformation of the adduct, while still corresponding to an essentially *gauche* arrangement $(-30°)$ with respect to α_5, is associated with $\alpha_4 = 180°$. The fixation of a cation at *B* has the effect of extending the structure of the polar head with respect to α_4. This effect is thus comparable to the influence of polyhydration [8].

Note that there is a relatively large zone in the neighbourhood of the minimum, within 2 kcal (for $\alpha_4 = 180°$, $-60° \leqslant \alpha_5 \leqslant 30°$ and for $\alpha_5 = -60°$; $-110° \leqslant \alpha_4 \leqslant -180°$).

Table 6. Interaction (kcal/mole) of Na^+ with EP[a] (site B; $\alpha_2, \alpha_3 = -81°, -81°$)

α_4	α_5	ΔE_1	ΔE_2
280	10	−37.0	17.8
260	10	−43.0	11.8
240	10	−46.2	8.6
210	10	−50.5	4.3
180	10	−52.2	2.6
150	10	−52.2	2.6
260	30	−46.0	8.8
180	30	−52.5	2.3
260	60	−48.6	6.2
240	60	−48.5	6.3
180	60	−52.0	2.8
270	120	−50.1	4.7
240	120	−45.1	9.7
180	120	−45.0	9.8
180	180	−48.4	6.4
180	240	−46.6	8.2
280	270	−47.7	7.1
180	270	−49.7	5.1
30	270	−45.7	9.1
300	300	−46.3	8.5
270	300	−51.3	3.5
260	300	−52.4	2.4
240	300	−53.7	1.1
180	300	−54.2	0.6
280	330	−47.2	7.6
180	330	−54.8	0

[a] $\Delta E_1 = E_{EP\ldots Na^+} - (E_{EP}$ (minimum) + $E_{Na^+})$; $\Delta E_2 = E_{EP\ldots Na^+} - E_{EP\ldots Na^+}$ (minimum).

Table 7. Interaction (kcal/mole) of Na^+ with EP[a] (site E; $\alpha_2, \alpha_3 = 180°, -81°$)

α_4	α_5	ΔE_1	ΔE_2
290	10	−28.8	26.7
280	10	−44.6	10.9
270	10	−48.9	6.6
260	10	−44.2	11.3
180	10	−16.8	38.7
270	20	−52.7	2.8
270	30	−55.2	0.3
270	40	−55.5	0.0
270	90	−55.2	0.3
270	120	−47.6	7.9
280	300	−44.2	11.3
280	330	−46.1	9.4
180	330	−26.1	29.4

[a] Definitions as in Table 6.

Site	α_4	α_5	ΔE_1
B	270	30	−87
B	180	180	−123
B	180	330	−126
E	270	40	−107
E	270	90	−113

Table 8. Interaction energies ΔE_1 in Ep . . . Ca^{++} (kcal/mole)

On the other hand, sodium binding at the *E* site has a different consequence. Table 7 indicates that the binding energies ΔE_1 conserve their highest value in the vicinity of $(\alpha_4, \alpha_5) = (270°, 30°)$, i.e. in the vicinity of the most stable conformation of free EP, and are appreciably smaller in the other explored regions. The examination of the molecular model shows that the low interaction energies found for the two conformations associated with $\alpha_4 = 180°$ should be conserved for other conformations with, or in the vicinity of, this value of α_4. As a result, the most stable conformation of the *E* adduct is very nearly the same as that for free EP, although its location is much less stringent: the minimum acquires a substantial degree of fluidity with respect to α_5, no significant variation of the conformational energy occurring for $30 < \alpha_5 < 90°$. This was not the case in free EP, where for instance the energy varies by 5 kcal/mole in passing from $\alpha_5 = 30°$ to $\alpha_5 = 60°$.

It is interesting that the global minimum found for Na^+ in position *E* of EP ($\alpha_4 = 270°$, $\alpha_5 = 40°$) with $\alpha_3 = -81°$, $\alpha_2 = 180°$, and the global minimum found for Na^+ in position *B* ($\alpha_4 = 180°$, $\alpha_5 = -30°$) with $\alpha_3 = -81°$, $\alpha_2 = -81°$, correspond to the same value of the binding energy of the cation ($\Delta E_1 = -55$ kcal/mole). In other words the interaction of Na^+ with the molecule of EP may occur equally favourably for two very different geometrical overall arrangements: either in position *E* with EP in a *trans-gauche-gauche-gauche* conformation (with respect to $\alpha_2, \alpha_3, \alpha_4, \alpha_5$), or in position *B* with EP in a corresponding *gauche-gauche-trans-gauche* conformation.

Concerning Ca^{++}, only a few representative conformations have been studied. The results are indicated in Table 8: they show an overall qualitative similarity with the trends manifested for the interaction with Na^+. The fixation in site *B* is energetically more favourable than in site *E*. The tendency to elongation of the structure to $\alpha_4 = 180°$ appears upon Ca^{++} binding in the *B* position as it did for Na^+ binding.

4. Conclusion

The present study, which involves the interaction of a single cation with one ligand (DMP^- or the polar head of phospholipids), is obviously a model study, experimental conditions, in particular in solution, corresponding generally to more complex situations. As such, it answers, however, a question frequently raised in connection with experimental studies on the conformation of this type of ligand in solution concerning the possible influence on their intrinsic conformation of the presence of cations, and in particular of the lanthanide cations used as paramagnetic probes in NMR studies. Although we did not deal with such cations in this paper (work is in progress on them), the present results

indicate that following the nature of the cation and the site of attachment, the binding may have a profound influence on the conformation of the ligand. This situation means that caution must be used when interpreting the corresponding experimental results. As an example of such a situation one may quote some recent investigations on the orientation of the polar head of phospholipids with respect to the bilayer surface. Thus a recent X-ray diffraction study on single crystals of phosphatidyl-ethanolamine obtained from glacial acetic acid has shown [24] the polar group to be approximately parallel to the plane of the bilayer, that is, perpendicular to the long-chain fatty acid residues (for a theoretical analysis of this case see [25]). A similar situation seems to exist in phosphatidylcholine vesicles [26] and in phosphatidylcholine-phosphatidylethanolamine mixed vesicles [27] studied by a $^{31}P[^{1}H]$ Nuclear Overhauser Effect. On the other hand a recent NMR investigation of phosphatidylcholine in bilayer vesicles using the lanthanide probes [28] points to a quite different conformation with the polar group approximately perpendicular to the bilayer plane. It seems to us quite plausible to consider that this situation is essentially the result of the cation binding to the phosphate group of the polar head. Such a binding may produce its effect through the extension of the phosphodiester group (which is *trans-gauche* in Ref. [28]), α_2-α_3 being fundamental in determining the parallel or perpendicular disposition of the polar head with respect to the bilayer surface. The further extension of the polar chain, suggested in Ref. [28], may partially be a consequence of this binding and partially due also to the effect of the solvent. The analogies in metal binding properties of Mg^{++} and lanthanides to *t*RNA [29] suggest that our results for this divalent cation may be quite illustrative of the situation with the trivalent rare earth probes.

The present results on the possible influence of cation binding on the conformation of the polar head of phospholipids argue also in favour of theories relating such changes to nerve excitation [30] or the negative steady-state resistance in excitable membranes [31].

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