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Quantum-Mechanical Studies of Environmental Effects on Biomolecules

VI. *Ab initio* Studies on the Hydration Scheme of the Phosphate Group

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Ab initio SCF computations using the STO 3G basis set have been performed on the hydration scheme of the dimethylphosphate anion (DMP^-) within the "supermolecule" approach. This model compound represents the phosphate group in nucleic acids, phospholipid components of membranes and a number of other important biological structures. In the first place the principal hydration sites for the fixation of a water molecule have been established and this was followed by the determination of the polyhydration scheme of the first solvation shell, which may involve up to six water molecules. The effect of hydration on the relative stabilities of the *gg*, *gt* and *tt* forms of DMP^- was evaluated. The study was extended to the computation of the energies of interaction in the second and third hydration shells and the conclusion was reached that organized hydration around DMP^- is essentially limited to two hydration shells with possible residual organization of a third shell around specific sites. The structure of the bound water was investigated. Charge transfer is observed from DMP^- to the bound water, the perturbation extending essentially to the first and with decreasing intensity, to the second hydration shell.

Key words: Hydration – Phosphate group – Nucleic acids – Phospholipids

1. Introduction

As an extension of a systematic series of theoretical investigations of solvation phenomena related to fundamental biological molecules and polymers (for review see [1]) we present here the results of *ab initio* computations of the hydration scheme of the phosphate group. This group is of an outstanding importance in a large number of biological compounds and in particular in the nucleic acids and the phospholipid components of membranes in which it represents one of the most important if not the most important site of interaction of the system with water.

2. Method

The dimethylphosphate anion (DMP^-) has been used as model compound. The geometry and the numbering adopted are recalled in Fig. 1. For easy recognition, the anionic oxygens carry the odd and the ester oxygens the even numbers.

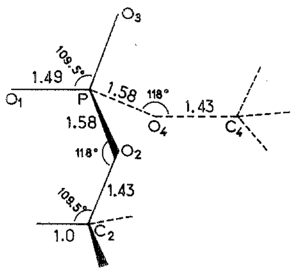

Fig. 1. Geometry and atom numbering utilized. (All OPO angles tetrahedral)

The geometry of both the ion and the water molecule were kept constant throughout the computations.

The procedure utilized is based on the "supermolecule" approach in which the interaction energies are calculated by comparing the energies of the adduct (DMP^- + water) with the sum of those of the isolated components [1]. Interaction with one molecule of water was considered first in all possible details so as to determine both the most favorable site of attachment and the lability of the binding with respect to various degrees of freedom. Next, the addition of further water molecules was considered until completion of the first hydration shell. Then an exploration was performed on the possible existence and properties of a second (and further) hydration shell(s).

The computations were carried out *ab initio* by the SCF LCAO procedure with an STO 3G Gaussian basis set [2], using the program Gauss 70 [3] and its extension [4] to accommodate 105 contracted functions. The *d* orbitals of the second-row atom have not been included in the present work. A separate investigation of the effect of their inclusion on the properties of the phosphate ion will be presented separately.

In the first stage of this work the phosphate group was maintained in the *gg* conformation about the P–O bonds, which is predicted by refined theoretical methods, SCF *ab initio* [5] and PCILO [6], to be its most stable conformation and which is also the conformation observed generally in the X-ray crystallographic studies of nucleic acids and their constituents (see e.g. [7, 8]) and of models of the polar head of phospholipids (for a review see [9]). This conformation corresponds to the values of ±60° for the torsion angles C_2–O_2–P–O_4 and O_2–P–O_4–C_4. (We recall that the torsion angle about the bond B–C in the sequence of atoms A–B–C–D is the angle through which the far bond C–D is rotated relative to the near bond A–B, the *cis*-planar position of bonds A–B and C–D representing the zero value. The torsion angles are considered positive for a right-handed rotation: when looking along the bond B–C, the far bond C–D rotates clockwise relative to the near bond A–B. Alternatively, the positive angles are defined as 0° to 180°, measured for a clockwise rotation and negative angles as 0° to −180°, measured for a counterclockwise rotation). In the second stage, aspects of the hydration scheme of the *gt* and *tt* forms of the phosphate have also been explored. These conformations correspond respectively to the values (60°, 180°) and (180°, 180°) for the two torsion angles under consideration.

Finally, we have considered some of the structural aspects of the water "bound" to the phosphate group in the hydration shells.

3. Hydration of the Phosphate in the *gg* Conformation

3.1. Monohydration

The results concerning the possibilities of hydration by one water molecule are best visualized by examining first the situation in the various OPO planes of the phosphate (Table 1 and Figs. 2a,b,c. The notations are explained in Table 1).

The plane O_1PO_3 contains the site corresponding to the *minimum minimorum* (−28.6 kcal/mole) on the energy hypersurface: this occurs in two equivalent positions when water is bound to one of the anionic oxygens, O_1 or O_3, by one H-bond, at the exterior of the OPO angle (positions E_{13} and E_{31}). But there is also, in that plane, another position E''_{13} (and its equivalent E''_{31}) only 1.2 kcal/mole less stable, where water is bound by one hydrogen to the other side of the anionic oxygen, with its second hydrogen turned towards the interior of the OPO angle. The corresponding position of water rotated by 180° about the H bond axis (E'_{13}) lies 4 kcal/mole above (*vide infra*). Moreover, there is the possibility of a bridge adduct B_{13}, nearly as favorable in energy (see Fig. 2a). This situation is comparable to that found in the case of the formate ion [11]. But, in contrast to this last case, there are here a number of other nearly as favorable binding sites in the other OPO-planes as shown in Table 1 and Figs. 2b and 2c: each OPO plane contains one external binding site E (to the anionic oxygen) similar to E_{13} and

Table 1. Monohydration of DMP^- (STO 3G)[a]

Plane	Symbol[b]	$d_{O_i \ldots H}$(Å)	θ	β	$-\Delta E$(kcal/mole)
O_1PO_3	E_{13} E_{31}	1.45	120	180	28.6
	B_{13}	1.75	110	143	27.1[c]
	E''_{13} E''_{31}	1.50	120	180	27.4
O_3PO_2	E'_{32}	1.50	120	180	26.7
	B_{32}	1.60	116	160	25.6
	E_{32}	1.50	120	180	25.1
O_1PO_4—equivalents: E'_{14} B_{14} E_{14}					
O_1PO_2	E'_{12}	1.45	120	180	27.0
	E_{12}	1.45	120	180	25.5
O_3PO_4—equivalents: E'_{34}, E_{34}					

[a] θ = angle between PO_i and $O_i \ldots H$ directions.
β = angle between $O_i \ldots H$ and HO_{water} directions.

[b] The symbols E_{ij}, E'_{ij}, E''_{ij} correspond to water bound by one hydrogen bond to oxygen i in the plane O_iPO_j. E stands for water external to the OPO angle, E' and E'' for water internal to this angle. In the unprimed and primed E_{ij}– positions the second hydrogen of water is turned towards the PO_i axis. In the double-primed position this second hydrogen is turned away from the PO_i axis (see Figs. 2a, b, c). The symbol B_{ij} corresponds to water making a bridge between oxygens i and j.

[c] Erroneously given as 26.8 in the preliminary report [10].

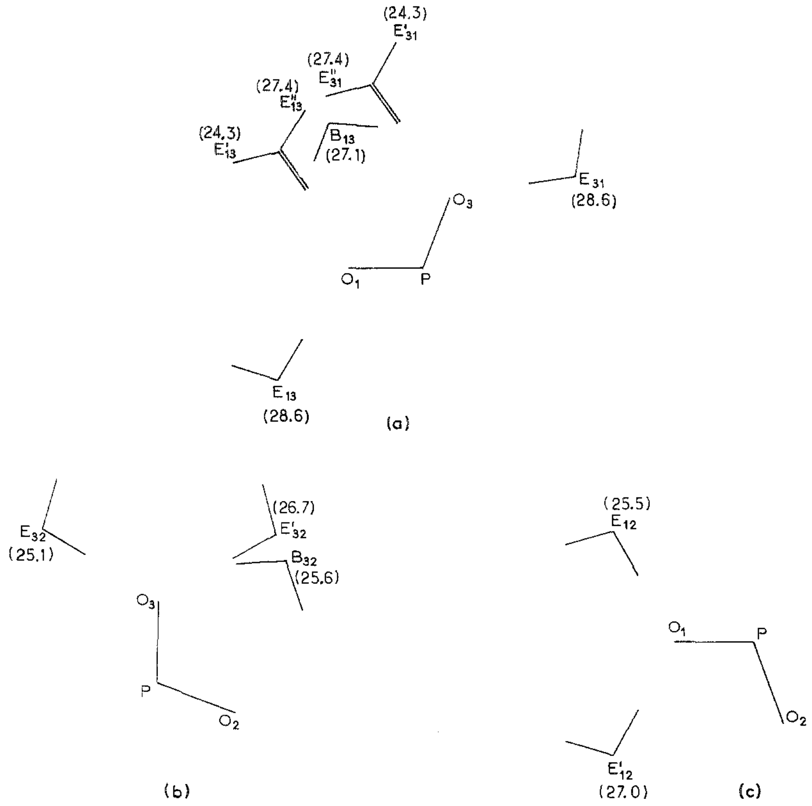

Fig. 2. Preferred hydration sites of the *gg* form in the planes: (a) O_1PO_3, (b) O_3PO_2 (equivalent: O_1PO_4), (c) O_1PO_2 (equivalent O_3PO_4), ($-\Delta E$ in kcal/mole)

E_{31}, and another site E' obtained from E by a 180° rotation about the PO axis. Moreover, a favorable bridged position B appears also in the equivalent planes O_1PO_4 and O_3PO_2, with an inclination of the water molecule towards the anionic oxygen, a situation reflecting the distribution of the electrostatic potential around the phosphate ion [12]. The corresponding bridge positions in the planes O_1PO_2 and O_3PO_4 are less favorable, which is another reflection of the potential distribution in these planes: the attractive character of the ester oxygens towards a proton is much smaller than that of the anionic oxygens and this is reflected in the smaller attraction of these oxygens for the second hydrogen of water and in the absence of individual binding positions of water at the esteric sites. This agrees with an observation by Sundaralingam [7] that the ester oxygen atoms are generally not observed in hydrogen bonding nor even in binding to metal ions. The exaggeration of the water binding capacity of the esteric oxygens in some of the empirical "solvation group" models is probably one of the factors responsible for the difficulties encountered by these models in their studies of the effect of water on the conformation of acetylcholine and related compounds [13].

On the whole, the distribution of the favorable hydration sites in the neighbourhood of the anionic oxygens points to the existence of a whole zone of attraction

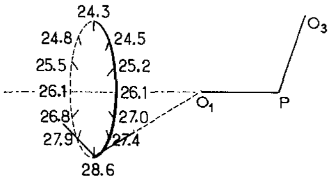

Fig. 3. Variation of $-\Delta E$ upon rotation of water out of position E_{13} ($O_1 \ldots HO$ sweeping a cone about the PO_1 axis with the non hydrogen-bonded hydrogen turned towards this axis)

for water about these two atoms: this has been confirmed by studying the variations in the binding energy obtained by rotating the plane of the water molecule about the PO_1 axis, from its most stable position E_{13}, in such a way that $O_1 \ldots HO$ describes a cone about this axis (Fig. 3). A circular zone of very large binding interaction with water was found, where the binding energy remains within 85% of its most favorable value. This zone corresponds to the circular zone of nearly constant attraction for a proton found in Ref. [12], the slight differences in the two distributions in these zones reflecting the obvious differences in the electrostatic attraction for a proton and a water molecule, and also the influence of the second-order delocalization effects which are included in the binding energy of water and are not included in the potential.

The flexibility in the binding of water to the phosphate moiety is further illustrated in Table 2 for two other possible movements of the solvent molecule away from its most stable position. It is seen that, as was observed in many other cases [1], the rotation about the H-bond axis is relatively easy: a rotation of 60° about this axis from position E_{13} decreases the binding energy only by 0.2 kcal/mole. On the other hand, the twisting of the water hydrogens out of the OPO plane in the bridged position is less easy, a situation also encountered in other cases [11, 14].

Table 2. Variation in the binding energy of water upon rotation in sites E_{13} and B_{13}

	$\theta°$	ΔE (kcal/mole)
	0	0
a	30	0.1
	60	0.2
	0	0
b	−10	0.4
	−20	2.1

[a] Rotation of the water molecule about the H-bond axis starting from position $E_{13}.d(O_1 \ldots H) = 1.45$ Å.

[b] Rotation of the water molecule about the PO_{water}-axis, starting from $B_{13}.d(O_1 \ldots H) = 1.75$ Å.

3.2. Polyhydration in the First Shell

The monohydration scheme outlined above, indicating the existence of large attractive zones of similar energies and many possibilities for the fixation of water, should result in multiple possibilities of polyhydration.

The results obtained for simultaneous fixation of a number of water molecules to the phosphate ion (distance = 1.8 Å for sites B and 1.5 Å for sites E, based upon optimization of model cases) confirm this expectation as shown by the examples given in Table 3. Up to six water molecules may be accommodated in the first hydration shell. (The addition of a 7th water molecule decreases the total binding energy). Intermediate hydration involving two to five water molecules may of course take place, a number of energetically nearly equivalent possibilities occurring for a given number of water molecules (3 for $n=2$ to 4, 2 for $n=5$). The average energy of water attachment is approximately -23 kcal/mole for $n=2$ and 3 and decreases when n increases further, down to -17.3 kcal/mole for $n=6$. The hydration scheme in each case may be easily visualized from the indications in Table 3 and the definitions of the symbols given in Table 1.

Experimental information about the hydration of the phosphate group comes mostly from studies of phospholipids. It is relatively abundant [15–23] and although it does not lead to a unique scheme and does not fix precisely the preferred sites of hydration, it indicates a number of "bound" water molecules which altogether is comparable with that suggested by the theoretical studies. Depending upon the experimental conditions and techniques utilized the number of water molecules in the primary hydration shell (most strongly bound) varies at the polar head of phosphatidylcholine (which was more abundantly studied than phosphatidylethanolamine) from 2 to 6. As in these molecules no strong hydrogen bond is expected around the cationic head, this number may be considered as relevant essentially to the phosphate group. (A secondary shell of 4–8 molecules of water, less strongly bound than the previous ones, is sometimes distinguished.) The

Table 3. Polyhydration of DMP$^-$ with n water molecules in the first shell

n	Occupied positions	$-\Delta E_{\mathrm{tot/n}}$ (kcal/mole)
2	$E_{13}\ E_{31}$	27.1
	$B_{14}\ E'_{12}$	23.6
3	$B_{13}\ B_{32}\ B_{14}$	23.0
	$B_{13}\ E'_{32}\ E'_{14}$	23.5
	$B_{13}\ E_{31}\ E_{13}$	24.7
4	$E_{13}\ E_{31}\ E_{14}\ E_{32}$	21.9
	$E'_{12}\ E'_{34}\ E'_{14}\ E'_{32}$	21.7
	$E_{14}\ E_{32}\ E'_{14}\ E'_{32}$	21.3
5	$B_{13}\ B_{32}\ B_{14}\ E'_{12}\ E'_{34}$	20.3
	$B_{13}\ E'_{32}\ E'_{14}\ E'_{12}\ E'_{34}$	20.3
6	$E_{31}\ E_{32}\ E_{34}\ E_{12}\ E_{13}\ E_{14}$	17.3

preferential fixation of 4–6 water molecules on the phosphates of the nucleic acids has similarly been proposed as a result of infra-red studies [24, 25].

4. Hydration of the Phosphate in the *gt* and *tt* Conformations

The *gg* conformation about the P–O bonds is as stated above the one predicted as the most stable for the free molecules of the type of dimethylphosphate or disugar phosphate or dinucleoside monophosphate or the polar head of phospholipids by refined quantum-mechanical procedures [5, 6, 8]. It is also the one observed predominantly for this type of compound in X-ray crystallographic studies, where the values of the corresponding torsion angles spread generally between 60°–90° or 270°–300° (for a review see [8]). The problem may, however, be raised as concerns the situation in solution for which less precise knowledge is available. As it is practically impossible, because of the complexity of the situation, to construct conformational energy *maps* for the hydrated species which would indicate the conformational possibilities as a function of the continuous rotation about the two P–O bonds, for different degrees of hydration, we have computed the essential features of the hydration scheme of the *gt* and *tt* forms and compared them with those of the *gg* form. In this way conclusions may be drawn at least about the effect of hydration on the relative stabilization or destabilization of these three typical forms.

For the *gt* form a complete study of the monohydration scheme with optimization for each position was done as for the *gg* case. The hydration sites being very little different from those of the *gg* conformer, the same symbols were kept. By extension, the same positions were also kept for the *tt* form.

Table 4 summarizes the essential results obtained. Its upper part indicates the values of the energies of binding for the attachment of one water molecule to the principal sites of hydration of the *gg* and *gt* conformers. The column $(gt-gg)$ indicates the energy difference between the two forms in the free dimethylphosphate anion and in its different monohydrates. It is seen that according to the site of the monohydration this difference, which is 3.4 kcal/mole in favor of the *gg* form in the free molecule, oscillates between the values of 2–3.9 kcal/mole in the monohydrates. Depending upon the position of its attachment, a water molecule may thus have a stabilizing or a destabilizing effect, although always a small one, upon the *gt* form with respect to the *gg* one.

A more regular evolution of the situation, indicated in the lower part of the Table, occurs in the polyhydrates. It is visible that both in the *gg* and in the *gt* form the binding energy per water molecule decreases with increasing hydration. The essential result, however, is that increasing hydration brings also the energies of the two conformers continuously closer to each other, to the point that when the first hydration shell is completed with its six water molecules, the two forms are practically equienergetical. For a lesser water content the *gg* form predominates but its excess of stability is less than it is in the free molecule.

Only one computation was carried out for the *tt* form and it is indicated in the last column of Table 4. In view of the preceding considerations it is quite illustrative. In the free molecule the *ab initio* computations indicate that the *tt*

Table 4. Hydration of the *gg*, *gt* and *tt* forms of DMP^-

n	*Symbol*[a]		*Energy*[b] (kcal/mole)						
			gg	*gt*		*gt*−*gg*		*tt*	*tt*−*gg*
	(a)	(b)		(a)	(b)	(a)	(b)		
0			0	3.4		3.4		8.0	8.0
1	B_{13}		−27.1	−27.6		2.9			
	E_{13}	E_{31}	−28.6	−29.6	−28.1	2.4	3.9		
	B_{32}	B_{14}	−25.6	−25.7		3.3			
	E'_{32}	E'_{14}	−26.7	−26.7	−28.1	3.4	2.0		
	E_{32}	E_{14}	−25.1	−25.9	−26.4	2.6	2.1		
	E_{12}	E_{34}	−25.5	−26.8	−25.7	2.1	3.2		
	E'_{12}	E'_{34}	−27.0	−27.6	−27.3	2.8	3.1		
2	$E_{13}\,E_{31}$		−27.1	−27.4		2.9			
3	$B_{13}\,E_{13}\,E_{31}$		−24.7	−25.0		2.6			
4	$E_{13}\,E_{14}\,E_{31}\,E_{32}$		−21.9	−22.2		2.2			
5	$\mathrm{B}_{13}\,E'_{12}\,E'_{14}\,E'_{32}\,E'_{34}$		−20.3	−20.7		1.1			
6	$E_{12}\,E_{13}\,E_{14}\,E_{31}\,E_{32}\,E_{34}$		−17.3	−17.9		−0.01		−18.3	1.8

[a] In the *gg* conformer, the positions (a) and (b) are equivalent.

[b] For $n=0$, the energy values are given with respect to *gg* taken as zero. For $n \neq 0$, the values in columns *gg*, *gt*, *tt* are $\Delta E_{tot}/n$ (hydration energy per water molecule); *gt*−*gg* and *tt*−*gg* is the difference in energy between the two conformers hydrated in the indicated positions.

form is 8 kcal/mole less stable than the *gg* form. We have computed the energy of the *tt* form hydrated with six water molecules and it appears that this form is then only 1.8 kcal/mole less stable than the *gg* form. It seems probable that the *tt* form with intermediate degrees of hydration will be relatively more unstable with respect to the other two forms.

The principal conclusion of this comparison is thus that polyhydration is destabilizing the *gg* form relative to the *gt* and *tt* forms. The *gg* form still remains, however, the most stable one for all degrees of hydration with the exception of hydration with six water molecules when the *gt* form becomes energetically equivalent to it. The *tt* form always remains the least stable one.

It may be pertinent to relate this theoretical result on the increase of the importance of the elongated forms of the phosphate group upon hydration to some recent observations on the conformation of purine and pyrimidine nucleotides in solution as studied by NMR spectroscopy in the light of theoretical computations of the relevant P–C spin-spin coupling constants in model systems. With the standard numbering system utilized in the study of mono- and polynucleotides (Fig. 4) it is the $^2J_{PC_{5'}}$ spin-spin coupling which may be related to the torsion angle about the P–$O_{5'}$ bond. In all non-cyclic mono- and polynucleotides studied the experimental values of this coupling are 4–5 Hz [26–28]. Theoretical computations on this coupling constant as a function of the torsion angle about the P–O

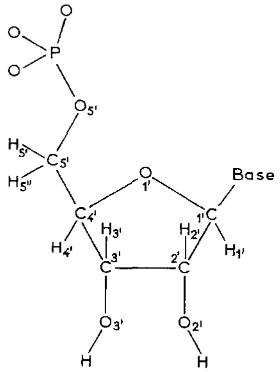

Fig. 4. Atom numbering in 5'-*β*-nucleotides

bond have been carried out by Giessner-Prettre and Pullman [29] on the model of ethyl phosphate: CH_3–CH_2–OPO_3^-. The results are given in Fig. 5, where they are presented for two values of the torsion angle about the C–O bond (0° and 180°) (For all intermediate values of this last torsion the curves will be located between the two given in the figure). The result shows that it is actually the torsion about the P–O bond which is decisive for the value of this coupling.

From the curves and the above quoted values of the $^2J_{PC_5'}$ coupling constants in mono- and polynucleotides it may be deduced that the torsion angles about their P–O bonds have values ranging between 120° and 150° (or 210° and 240°, NMR not permitting the distinction between the two cases). This result suggests

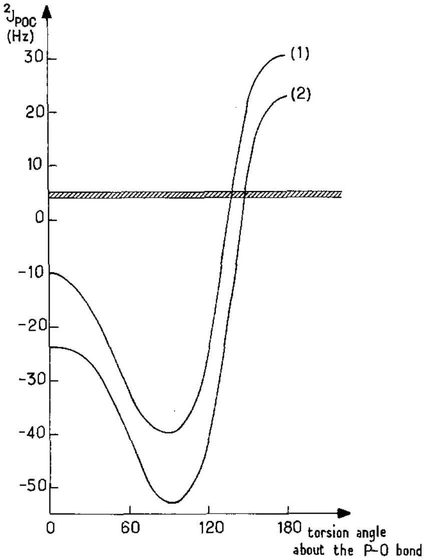

Fig. 5. Computed variation of $^2J_{POC}$ with the torsion angle about the PO bond. Torsion angle about the C–O bond equal to (1): 0°; (2): 180°. The horizontal line indicates measured values of *J* for nucleotides

strongly that the torsion angles about the P–O bonds may have values in solution which are different from those measured in the crystal phase or that there is an equilibrium in solution between several conformers corresponding to different values of these torsion angles, the average conformation having a somewhat larger value for this angle than has been observed in crystals in most cases.[1] These results bear obviously a close relationship to our computations indicating a possible elongation of the structure of the phosphate group promoted by hydration.

A related effect was put into evidence in a theoretical study of the influence of hydration on the conformation of the polar head of phospholipid components of membranes [32].

5. The Second Hydration Shell and beyond

The next step of our research consisted of going beyond the study of the first hydration layer and of exploring the possibility of existence of organized more remote layers, in particular of the second one. The problem raises from the start two practical questions.

1. The modalities of coordination of the water molecules of the second and further hydration shells with those of the preceding shell and among themselves. It is frequently admitted that water binds to biological substrates through tri- and tetracoordination [33]. A large number of theoretical studies on the structure of water oligomers [34–40] and on the interaction of water with simple anionic systems (e.g. OH^-) [41] show the possibility of both arrangements which do not differ much in energy, with perhaps some preference for tri-coordination. We have explored the possibilities of forming the second (and next hydration shells) around DMP^- both through tri- and tetracoordination of water molecules of these shells to those of the first.

2. The determination of the extent of the hydration surface around DMP^- necessitates a better evaluation of the significance of the computed values of the interaction energies than does the mere determination of the overall structure of the first water layer. Thus it is well known that the STO 3G basis overestimates the binding energies and allows too close an approach of a water molecule to ionic species [42, 43], although the qualitative features of the binding are correctly reproduced (see Refs. [43] and [44] for a discussion). In view to estimate the influence of this situation upon the evaluation of the interaction energies between successive hydration shells, a model *ab initio* computation was performed in the STO 3G basis on the hydration energies of the adducts of $OH^- \ldots (H_2O)_n$ and the results compared with those obtained for the same system by Newton and Ehrenson [41] using the more refined 4-31 G basis set.

The results shown in Table 5 are very instructive. They indicate that while the overestimation of the energy of interaction of a single water molecule with OH^-

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[1] Some dinucleoside monophosphates in the solid state manifest values of one of their P–O torsion angles close to 150° ($U_{3'}P_{5'}A$, molecule 1 with a value of 163° Ref. [30]) or 240° ($A_{2'}P_{5'}U$, with an angle of 233°, Ref. [31]), reminiscent of the effect in solution.

Table 5. Energies of hydration of $(H_2O)_n \ldots OH^-$

Configuration	4–31G basis (Ref. [41])	STO 3G basis
	ΔE^a	ΔE^a
Trigonal		
One water molecule in the first shell	−40.7	−69.8
Two water molecules in the first shell	−30.1	−36.6
Two water molecules in the first shell + one in the second shell	−20.1	−22.3
Tetrahedral		
Three water molecules in the first shell	-23.1^b	-23.4^b
Three water molecules in the first shell + one in the second shell	−15.9	−16.8

[a] $\Delta E = E[OH^- \ldots n(H_2O)] - E[OH^- \ldots (n-1)H_2O] - E[H_2O]$ (in kcal/mole).
[b] Energy per water molecule.

by the STO 3G basis set with respect to the 4-31 G basis set is very appreciable (of the order of 70%), the discrepancy diminishes very rapidly with the increase of the number of the water molecules to the point of becoming nearly insignificant already for hydration with three water molecules. A similar situation was observed for progressive solvation of NH_4^+ by water and by ammonia where, moreover, the criterion was not the agreement with more refined computations but with experimentally measured enthalpies [45]. Under these circumstances it may be assumed that STO 3G computations may trustfully be expected to yield results as reasonable as those of the split basis for the problem which we are investigating presently and which involves a number of water molecules.

The study of the characteristics of the second and further hydration shells of DMP⁻ was in fact performed in two stages. The first one consisted of a broad attempt to delineate the general gross features of the constitution of these shells. For this sake, a water molecule was fixed in one of the typical preferred hydration sites of the first hydration shell: B_{13}, E_{13}, E_{31}, B_{32}, E'_{32}, E'_{34}. The fixation of a second water molecule upon the first one was then investigated by approaching it with one of the hydrogen atoms in the plane and along the bisectrix of the HOH angle of the first water molecule. An equilibrium distance of 1.5 Å, found by approximate optimization of the adduct upon the B_{13} bridge was adopted as typical for the water molecules of the second hydration shell. The procedure was then extended to water molecules in the next hydration shells.

The results for successive stepwise linear addition (tricoordination) of water molecules to the principal sites of the first hydration shell, computed in this approximation are indicated in Table 6. For some of them the addition has been carried out for the *gg* and *gt* conformations of DMP^-. It is found that as an average the interaction energies of the water molecules of the second hydration shell represent about 60% of the interaction energies of the water molecules of the first shell. It is seen also that water of the second shell is preferentially structured around the B_{13} site and next around the E_{31}, E_{13} and B_{32} sites. This succes-

Table 6. Hydration energy ΔE^a of $DMP^- \ldots n(H_2O)$ (one H_2O molecule in each shell, linearly bound) (kcal/mole)

Site of location of the first water molecule	Conformation of DMP^-	1st shell	2nd shell	3rd shell
B_{13}	*gg*	−27.1	−17.6	−12.1
	gt	−27.6	−17.7	
E_{13}	*gg*	−28.6	−14.0	−10.0
	gt	−29.6	−13.8	−9.5
E_{31}	*gt*	−28.1	−14.6	−10.0
B_{32}	*gg*	−25.6	−14.3	
E'_{32}	*gg*	−26.7	−13.4	
E'_{34}	*gg*	−27.0	−11.0	

[a] Same definition as in Table 5.

sion of preferences is not identical with that of the first hydration shell. It may also be observed that the energies of water interaction in the second shell are practically independent of the *gg* or *gt* conformation of DMP^-. Table 6 shows also that the energies of interaction of the water molecules of the third hydration shell represent in this approximation about 35–40% of the energies of hydration of the first hydration shell.

It may be interesting to indicate the impossibility to fix a water molecule of the second hydration shell through its oxygen atom on the terminal hydrogen atom of the water molecule of the first hydration shell. The energy computed for such a system is higher than that of the sum of the separated components at least for $d_{H\ldots O} < 15$ Å. This result may be understood through the examination of the charge distribution in this system (*vide infra*): the water molecule of the first hydration shell carries a net negative charge of $\simeq 0.1$ e which exerts a repulsive effect upon the nucleophilic oxygen of the second approaching water molecule. A similar inability to act as proton donor has been found in reference [41] for a water molecule bound to the hydroxyl ion.

The results discussed above relate, as stated, to *single* water molecules placed on each hydration shell. Even within the general simplified assumptions adopted above, a more precise evaluation of the values of the mean energies of interaction characteristic of the different shells may be expected from computations on polyhydration, involving simultaneously a larger amount of water molecules. Three typical results are presented in Table 7. The fundamental indication is that polyhydration diminishes the mean energy of interaction of the water molecules of each hydration shell. Thus let us consider a hexahydrate of DMP^- composed of three water molecules in the first hydration shell at sites B_{13}, E_{13} and E_{31} and three other water molecules bound linearly (trigonally), one to each of the preceding ones. The mean value of the individual hydration energy of the three water molecules of the first shell is −28.1 kcal/mole; it becomes −24.7 kcal/mole in the corresponding trihydrate. Similarly the mean value of the individual hydration energies of the three water molecules of the second hydration shell which is −15.2 kcal/mole drops to −12.9 kcal/mole in the polyhydrated second shell. It

Table 7. Hydration energies of polyhydrated DMP$^-$ (kcal/mole)

Number of water molecules	Location	Hydration energy of the 2nd shell	Hydration energy per water of 2nd shell	Hydration energy of the 3rd shell	Hydration energy per water of 3rd shell
6	Three in the first shell: B_{13}, E_{13}, E_{31} three linearly in the second shell	−38.7	−12.9		
3	One in the first shell: B_{13} two tetrahedrally in the second shell	−30.8	−15.4		
7	One in the first shell: B_{13} two tetrahedrally in the second shell four tetrahedrally in the third shell			−32.3	−8.1

may be deduced from this situation that the individual values of the hydration energies of the water molecules of the 3rd shell which are of the order of −10 kcal/mole, will similarly become reduced in a polyhydration scheme to values not much greater than the energy of dimerization of water itself (−6.4 kcal/mole in the STO 3G basis). It seems therefore relatively improbable that organized "bound" water layers may extend around DMP$^-$ beyond the 2nd shell, although residual organized such fragments may persist in the vicinity of some particularly favorable hydration sites.

Table 7 presents also two results of polyhydration involving tetrahedral coordination of the water molecules of the second and third hydration shells. The value of −15.4 kcal/mole obtained as the mean value of the energy of interaction for the two water molecules fixed in the second hydration shell on a water molecule in position B_{13} of the first shell may be compared with the value of −17.6 kcal/mole found in Table 6 for a single water molecule fixed trigonally in the second shell at the same site. The decrease of the mean energy of interaction for the water molecule fixed tetrahedrally in the third hydration shell (last line of Table 7) is worth stressing.

In an attempt to refine quantitatively the information gathered in the preceding exploration, one larger computation was carried out involving a complete first hydration shell of six water molecules plus one water molecule of the second shell added to the first one at site E_{13}. Moreover, the distance between the two water molecules at that site was reoptimized (and found to be equal to 1.7 Å). In this computation the energy of interaction of the water molecule of the second shell was found to be equal to −11.9 kcal/mole, a value significantly smaller thus than that of 14 kcal/mole found (Table 6) when two water molecules were considered alone at the E_{13} site. The completion of the first hydration shell has thus the effect of reducing the binding energy of the water molecules of the second hydration shell and this effect extends doubtlessly to further shells. This result strengthens the conclusion that organized "bound" water is most probably essentially limited to the two first hydration layers around DMP$^-$.

6. The Structure of "Bound" Water

The last subject which we would like to discuss concerns the effect produced on the properties of the "bound" water molecules by the organization of the hydration shells. This can be visualized by the distribution of the electronic charges obtained by a Mulliken population analysis performed on the wave functions. The most representative results in this respect are given in Figs. 6–10.

Fig. 6 presents the distribution of the net electronic charges in free DMP$^-$ and water. The absence of *d* functions on the phosphorus atom results in a strong exaggeration of the numerical values of the net charges on the phosphate ion [46]. One should also keep in mind the fact that the utilization of an STO 3G basis set leads to an overestimation of the charge transfer [43]. However these aspects of the problem are of secondary importance in the comparative studies that we wish to carry out here. The same figure contains also the distribution of the electronic charges in the first hydration shell of the two monoadducts B_{13} and E_{31} at their equilibrium O...H distances.

It may be observed that the structure of the "bound" water is significantly perturbed with respect to that of free water: its oxygen atom has become appreciably more negative and its hydrogen(s) engaged in the hydrogen bond(s) with DMP$^-$ have become significantly more positive. Concomitantly the phosphorus of DMP$^-$ has increased its net positive charge while its anionic oxygens have undergone a decrease of their negative charges. Altogether the binding of water

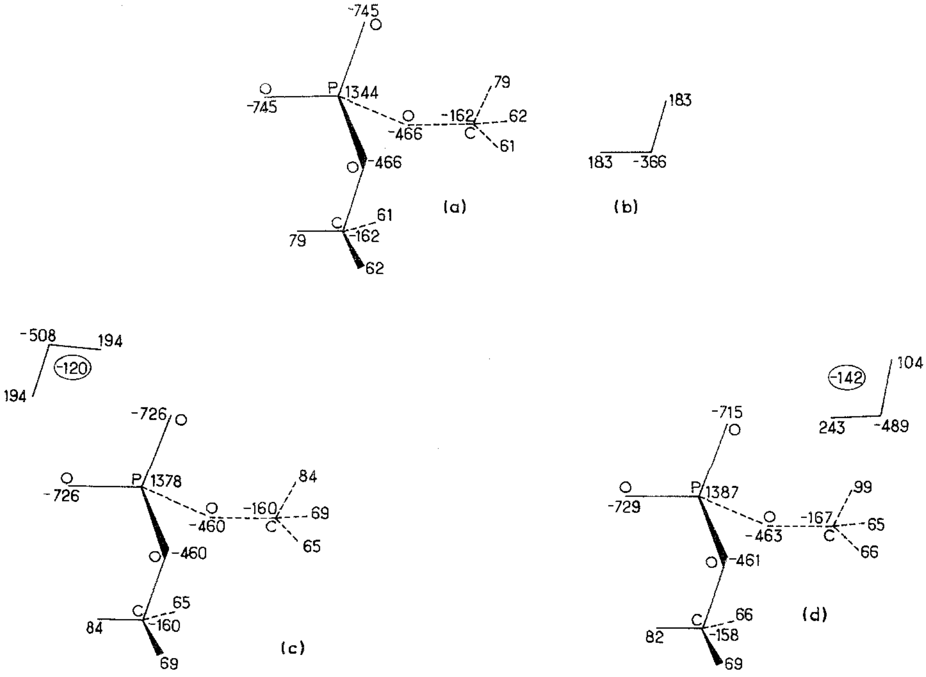

Fig. 6. Net charges (in millielectron units) in (a) DMP$^-$, (b) water, (c) B_{13} hydrate, (d) E_{31} hydrate. The numbers in circles indicate the global net charge on the water molecule

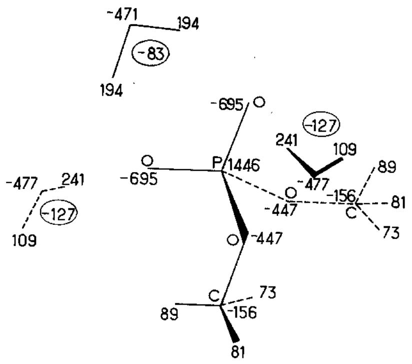

Fig. 7. Net charges (in millielectron units) in the B_{13}, E'_{14}, E'_{32} trihydrate of DMP$^-$. The numbers in circles indicate the global net charge on the water molecule

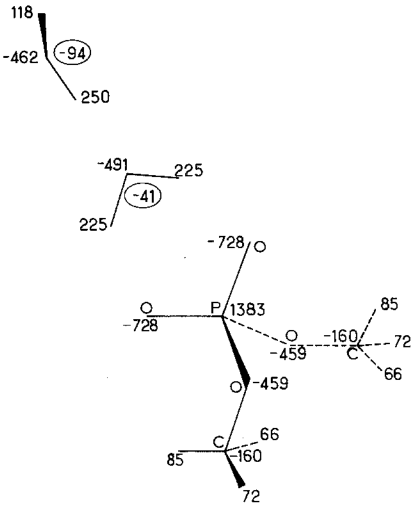

Fig. 8. Net charges (in millielectron units) in a dihydrate of DMP$^-$ (one water molecule in the first hydration shell and one in the second). The numbers in circles indicate the global net charge on the water molecule

to DMP$^-$ produces a partial transfer of electrons from the latter to the former, the transfer is greater (0.142 e) in case of the non-symmetrical binding E_{31} than in case of the symmetrical binding B_{13} (0.120 e). The bound water carries thus an excess of electronic density. The situation may be compared to the case of water dimers or polymers in which there is already a small charge transfer from the proton acceptor to the proton donor molecule [34, 35] and to the case of the interaction of water with OH$^-$ [41]. The charge transfer in the case of DMP$^-$...H_2O (and similarly of OH$^-$...$(H_2O)_n$ [41]), is more important than in the case of the water dimer where an STO 3G calculation indicates a value of 0.039 e. This enhancement of the charge transfer is a result of the anionic nature of the solute which transmits a small partial anionic character to the bound water molecule of

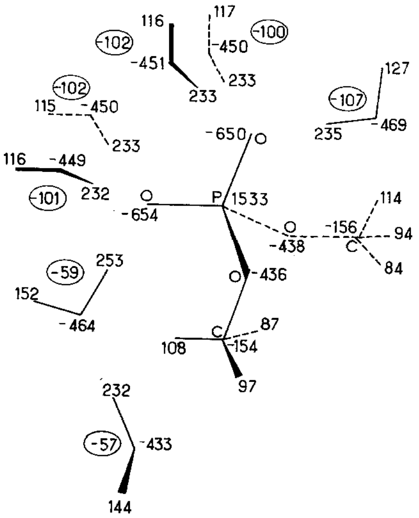

Fig. 9. Net charges (in millielectron units) in a heptahydrate of DMP⁻ (six water molecules in the first hydration shell and one in the second). The numbers in circles indicate the global net charge on the water molecule

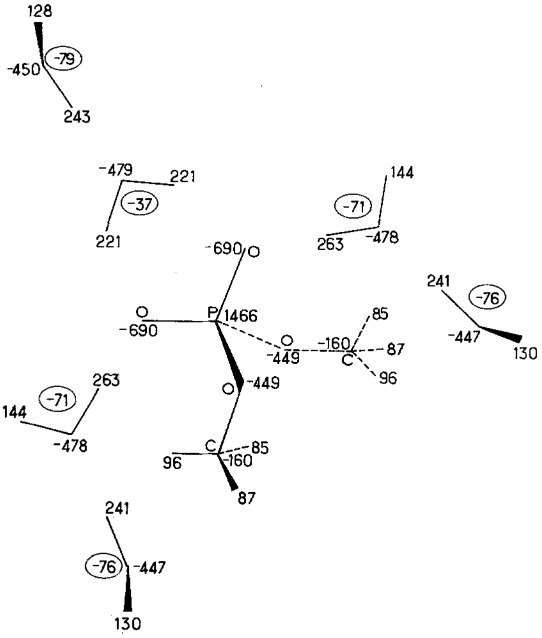

Fig. 10. Net charges (in millielectron units) in a hexahydrate of DMP⁻ (three water molecules in the first hydration shell and three in the second). The numbers in circles indicate the global net charge on the water molecule

the first layer, thereby enhancing its attraction towards other water molecules: note the increased negative character of the oxygen of the bound water in Figs. 6c and 6d, compared to isolated water.

Polyhydration in the first shell, while confirming these general features indicates that the presence of a number of water molecules reduces in each of them the amount of charge transfer found for a single molecule. This is illustrated in Fig. 7 which presents as an example the distribution of the electronic charges in the trihydrate B_{13}, E'_{14}, E'_{32} of DMP$^-$ (all water molecules in the first hydration shell).

New features appear in the structure of water when solvation is extended to the second hydration shell. They are illustrated in Figs. 8–10.

In Fig. 8 there is one water molecule in the first shell and one in the second. In Fig. 9, the first shell is completed to 6 molecules of water, with one water molecule in the second shell. Fig. 10 corresponds to three molecules in the first shell and three in the second shell. Let us denote them 1/1, 6/1 and 3/3 respectively.

The most conspicuous features appearing in comparing Fig. 8 (1/1) with Fig. 6d is that the overall transfer of charge from DMP$^-$ to the water molecules takes place predominantly at the benefit of the terminal molecule: thus the excess of charge of the water molecule of the first hydration shell is only 0.041 e while that of the water molecule of the second hydration shell amounts to 0.094 e. This last number is nevertheless smaller than the 0.120 e transferred to the single water molecule of Fig. 6c, indicating a decreasing ability of the water molecules of the second shell for further binding. This is equally shown by the decrease in charge on the oxygen of the terminal water (-0.462 instead of -0.508 in Fig. 6c).

A more complete and significant picture may be observed in the heptahydrate (6/1) of Fig. 9. It appears here that the transfer of electronic charges leads to an excess of approximately 0.1 e at each water molecule of the first hydration shell, with the exception of the molecule in site E_{13} (bound to the water of the second shell), where the excess is smaller. Furthermore, on the water molecule of the second shell, the charge transfer amounts only to 0.057 e. Similarly to the situation in case 1/1, the negative charge on the oxygen atom of the water molecule of the second shell has decreased to -0.433.

This redistribution of charge transfer over the successive layers of bound water is particularly evident in Fig. 10 (3/3).

It may thus be estimated that in a reality which involves the completion of rather large hydration shells the polarization of the water molecules due to the effect of the perturbing anionic solute will decrease in successive solvation layers. The decrease may be estimated as relatively rapid and it seems probable that the water molecules of the third hydrated shell around DMP$^-$ will be only very slightly perturbed if at all. Compared with the 1/1 data of Fig. 8, this result shows the necessity of taking into account the totality or at least large parts of the first hydration shell in drawing conclusions about the characteristics of the second layer. It also agrees with the conclusion based on energy computation that "bound" perturbed water around DMP$^-$ is essentially restricted to the first two hydration shells.

Conclusions

The aim of this work was to present within the supermolecule model a picture of the hydration scheme of DMP$^-$, the fundamental component of a number of biological structures, which is also one of their essential hydration centers. Because of the relatively large number of water molecules involved in the hydration scheme and the utilization for practical reasons of a simple basis set, the results of the computations have to be considered as a semi-quantitative representation of the situation. As indicated in the text, however, the deficiencies of the STO 3G basis set are much less pronounced in the study of polyhydration than for monohydration and attempts have been made continuously by us to include as much as possible the mutual interaction between the hydration sites and shells. It is therefore expected that the overall picture obtained is significant.

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