Calculation of electrostatic interactions in torsional potential of the internucleotide phosphodiesteric unit

[a]J. MLÝNEK, [a]T. BLEHA, and [b]I. TVAROŠKA

[a]*Polymer Institute, Slovak Academy of Sciences,*
809 34 Bratislava

[b]*Institute of Chemistry, Slovak Academy of Sciences,*
809 33 Bratislava

The effect of parametrization of the electrostatic term on the conformational energies of a phosphodiesteric unit has been investigated by the method of potential functions (PFM). The charges found for the dimethyl phosphate ion by different quantum chemical methods have been used as parameters. It has been revealed by using the CNDO/2 or *ab initio* charge distribution that the electrostatic term nearly does not contribute to the torsional potential around the P—O bond. In this case, PFM predicts energetically almost equivalent *gauche* and *trans* stable conformations for the rotation around the P—O bond. The inclusion of the lone electron pairs on oxygen atoms into the electrostatic term does not result in the expected suppression of preference of the *trans-trans* conformation, but on the contrary, it favours considerably this conformation with respect to the others. A comparison of the torsional potentials obtained by CNDO/2 and PFM methods has revealed that the PFM does not involve those intramolecular interactions the symmetry of which can be described by the terms V_1 and V_2 in the Fourier expansion of torsional potential. Attention was also paid to other approximations inherent to PFM that may be the origin of differing predictions for oligo- and polynucleotides given by PFM on the one hand and MO calculations and X-ray data on the other hand.

При помощи метода потенциальных функций (МПФ) изучался эффект параметрирования электростатического члена конформационных энергий фосфодиэфирной группы. В качестве параметров были использованы заряды диметилфосфатного иона, найденные различными квантовохимическими методами. С использованием CNDO/2 или *ab initio* методов для вычисления распределения заряда было найдено, что электростатический член не влияет на торзионный потенциал связи Р—О. В этом случае МПФ приводит к энергетически почти эквивалентным *гош-* и *транс-*стабильным конформациям при вращении вокруг связи Р—О. Учет свободной электронной пары кислородного атома в электростатическом члене не ведет к ожидаемому подавлению населения *транс-транс* конформации, а,

наоборот, к ее существенному предпочтению перед другими. Сравнение торзионных потенциалов полученных при помощи методов CNDO/2 и МПФ показало, что МПФ не включает такие междумолекулярные взаимодействия, которых симметрия описывается членами V_1 и V_2 ряда Фурье для торзионного потенциала. Уделялось внимание также другим приближениям ингерентным к МПФ, которые могут являться причиной различий в предпосылках относительно олиго- и полинуклеотидов, вытекающих, с одной стороны, из МПФ и, с другой стороны, из расчета МО и рентгеновских измерений.

The method of potential functions (PFM) is successfully used for theoretical predictions of three-dimensional structure of more complicated biological molecules and macromolecules. In this method, the total potential energy of molecule V is additively expressed by several contributions such as nonbonding interactions V_{NB}, "intrinsic" torsional barriers to internal rotation around single bond V_{TOR}, electrostatic interactions in molecule V_E, energy of intramolecular hydrogen bonds V_H, *etc.*

$$V = V_{NB} + V_{TOR} + V_E + V_H \ldots \qquad (1)$$

Owing to its relative simplicity, the PFM method enables us to calculate even more complicated molecules the size of which exceeds the present practical limitation of quantum chemical methods. The PFM method was frequently used for elucidation of the conformational structure of nucleic acids and their components [1—5]. As PFM is a semiempirical method involving many parameters, the quality of its results depends on the reliability of the parameters used. The phosphodiesteric unit in a chain of nucleic acids has no analogy among other biological macromolecules in consequence of which the reliability of the PFM parameters for this bond segment is relatively low and additional careful testing is necessary. As to the calculation of nonbonding interactions, a general procedure [6] usable even for the phosphorus atoms is available, but the situation is much worse as regards the parameters for the terms V_{TOR} and V_E. In this respect, the rather arbitrary values published in the first paper on theoretical conformational analysis of the phosphodiesteric unit [7] are almost generally accepted.

This rather unsatisfactory situation is reflected in some respects in the disagreement between the PFM results obtained for oligo- and polynucleotides on the one hand and quantum chemical molecular-orbital calculations and X-ray structural data on the other hand. A typical example is the PFM prediction of a preference of the stretched *trans-trans* conformation of the phosphodiesteric unit which was questioned [8, 9] because it has not been confirmed experimentally and quantum chemical calculations do not indicate its energetic preference in comparison with *gauche-gauche* or *gauche-trans* conformations. This contradiction is still more disagreeable since on the basis of rigid body conception [10] the rotation around

the P—O bonds is the most significant factor which determines the flexibility and overall spatial arrangement of nucleic acids.

The aim of this study is to investigate whether the reliability of PFM in the description of torsional potential around the P—O bond could be enhanced by reparametrization of the term V_E on the basis of the charge distribution obtained by the use of quantum chemical methods more sophisticated than the method originally applied [7]. We have also investigated how the inclusion of the lone electron pairs on the oxygen atoms manifests itself in conformational dependence of the term V_E.

Model and methods

We used the dimethyl phosphate anion (DMP⁻) CH_3—O—PO_2—O—CH_3^- as the simplest model of the phosphodiesteric unit in polynucleotides. We assumed a rigid geometry of molecule, independent of rotation with the structural data given in Fig. 1. We investigated the rotation around the P—O bonds characterized by the dihedral angles ω and ω'. The latter become 0° for *cis* position of the atoms $O_e PO_e C$, 60° for *gauche* (G) position, and 180° for *trans* (T) position. The methyl groups were in staggered position so that the hydrogen atoms H_t were *trans*-oriented with respect to the atom P.
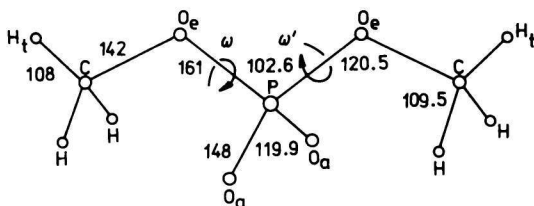

Fig. 1. Molecule of DMP⁻ in TT conformation, $\omega = \omega' = 180°$.
Bond lengths in pm, bond angles in degrees.

In calculating the contributions to the potential energy of molecule according to eqn (*1*), we used the parameters and form of potential functions according to [11] for the term V_{NB} and in harmony with usual procedure, we approximated V_{TOR} by means of a term with triple barrier in the form $V_{TOR} = (V_0/2)(1 - \cos 3\omega)$ and assumed that $V_0 = 4.186$ kJ mol⁻¹ [7, 11]. Above all, we were interested in obtaining the electrostatic contribution. It is usually calculated on the basis of the Coulomb law

$$V_E = \sum_{i<j} q_i q_j / \varepsilon \, R_{ij} \qquad (2)$$

in the so-called monopole approximation where q_i and q_j are net charges localized on atoms in a molecule. As the calculations concern an isolated molecule, we have assumed a unit value for the dielectric constant ε.

Besides the calculations based on PFM, we also investigated DMP$^-$ by the semiempirical quantum chemical method CNDO/2 in standard parametrization [12] with the both *sp* and *spd* phosphorus basis sets. CNDO/2 results for DMP$^-$ were used for the determination of V_E by means of eqn (*2*) as well as for the comparison of the forms of torsional potential obtained by the PFM and CNDO/2 methods for the P—O bond.

Results and discussion

Torsional potential using the net charges on atoms in the V_E term

The values of net charges q_i calculated by the CNDO/2 method for DMP$^-$ in TT conformation with the *sp* as well as *spd* basis sets are listed in Table 1. It is known that the CNDO/2 method gives a relatively reliable picture of the charge distribution in a molecule. That can be also confirmed by a comparison with the *ab initio* results taken from [13]. The two methods give consistently overestimated phosphorus and oxygen atomic charges unless the *d* orbitals are included in the basis set. Both methods indicate consistently that the negative charge of anion is localized in the central PO$_2^-$ group, but they give different results as regards the polarity of the charge localized on the carbon atom and the hydrogen atoms in the methyl groups.

Table 1 presents also charges obtained as a sum of π and σ charges that were calculated by the Hückel and Del Re methods [7]. This approach is still used for the determination of V_E in biological phosphates. We have assumed that the use of a more accurate charge distribution given by more sophisticated quantum chemical calculations of the CNDO/2 and STO-3G types should provide a more reliable picture of the intramolecular electrostatic interactions in a phosphodiesteric unit.

By using eqn (*1*) and the above-mentioned parameters, we calculated the PFM maps of conformational energies for the rotation with respect to ω and ω' angles. In these calculations we employed the charges q (Table 1) determined for the TT conformation of DMP$^-$ since their variation with rotation is negligible. Unexpectantly, the character of the conformational energy maps varies only very little with the use of different CNDO/2 and *ab initio* charges taken from Table 1. For both the *sp* and *spd* set of charges, symmetrical conformational energy maps are obtained. They are relatively flat with nine inconspicuous minima of the TT, TG, and GG type which energetically differ only little from each other. In all four cases, the most stable conformation of DMP$^-$ is the doubly degenerated GG conformation (G$^+$G$^+$ and G$^-$G$^-$) with the opposite orientation of the CH$_3$ groups with respect to the O$_e$PO$_e$ plane. The next one is always the TT conformation, followed by the fourfold degenerate TG conformation and the G$^+$G$^-$ and G$^-$G$^+$ conformations. The relative energies of the TT and TG conformations referred to the GG conformation for different sets of charges are also given in Table 1.

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Table 1

Values of charges on atoms in DMP$^-$ found by different quantum chemical methods and the PFM conformational energies calculated by means of these values (charges in e.u., energies in kJ mol^{-1})

	O_a	O_e	P	C	H	H_t	$V_{TT} - V_{GG}$[c]	$V_{TG} - V_{GG}$[c]
CNDO/2 sp*	−0.612	−0.470	1.218	0.146	−0.056	−0.062	1.00	1.17
spd*	−0.441	−0.291	0.389	0.168	−0.034	−0.058	1.26	1.31
ab initio** sp^a	−0.757	−0.477	1.360	−0.147	0.067	0.067	1.16	1.21
spd^b	−0.511	−0.349	0.534	−0.129	0.074	0.074	0.93	0.99
Empirical***	−0.830	−0.213	0.802	−0.039	0.060	0.060	−9.40	−4.49

* This paper.
** Ref. [13].
*** Ref. [7].
  a) STO-3G basis set.
  b) STO-3G basis set with inclusion of d functions.
  c) Energies calculated according to PFM in this study by using corresponding charges.

By a more detailed analysis of the conformational energy maps, we have found that their overall form approximately corresponds to the sum of the terms V_{NB} and V_{TOR}, which means that the term V_E only very little contributes to the torsional dependence of potential energy. This may be demonstrated by an intersection through conformational maps for $\omega = 180°$ (Fig. 2). The change in total potential
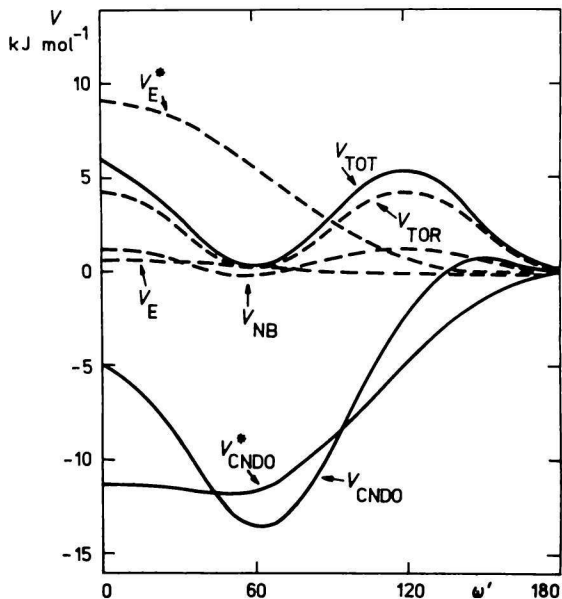

Fig. 2. Variation of the total energy V with the angle ω' while ω is fixed at $\omega = 180°$ (sections through conformational energy maps). V_{TOT}, V^*_{CNDO}, and V_{CNDO} are total potential energies of DMP$^-$ obtained using the PFM, CNDO/2 *sp*, and CNDO/2 *spd* methods, respectively. The curves V_{TOR}, V_{NB}, and V_E represent the intrinsic torsional, nonbonding, and electrostatic components of the energy V_{TOT}, respectively. V_E is calculated by using the CNDO/2 *spd* charges, V^*_E term using charges from [7].

energy of DMP$^-$ due to rotation by ω' is expressed by the curve V_{TOT} while the electrostatic contribution V_E has been obtained by the use of the CNDO/2 (*spd*) charges. Analogous courses of the term V_E which are almost independent of conformation have been obtained for other sets of charges, too. The rotational independence of the term V_E may be explained if we realize that the position of the central PO$_2^-$ group where the substantial portion of the electron density in a molecule is concentrated does not change owing to the rotation by the angle ω' and only rather small charges on atoms in the CH$_3$ group rotate. Moreover, the individual charges on atoms of the methyl group are of opposite sign and thus nearly compensate each other so that the effective charge of CH$_3$ group is very small.

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Therefore it appears that the contribution of V_E to the torsional potential around the P—O bond is affected mainly by the character of charge distribution in the methyl group which is approximately described by the effective charge of the CH_3 group. This conclusion is also confirmed by comparing the conformational energy maps calculated from the CNDO/2 and *ab initio* charges with the map determined on the basis of the charges hitherto usually used for a phosphodiesteric unit (Table 1) [7]. Since we use the geometry of DMP⁻ as well as the form of potentials for V_{NB} somewhat different from that in [7], the calculated map slightly differs from the original ones [7], nevertheless it predicts much greater stabilization of TT conformation with respect to TG and GG conformations than maps with CNDO/2 and *ab initio* charges. This difference is due to different character of the conformational dependence of the electrostatic term calculated using charges from [7], which is documented by the curve V_E^* in Fig. 2. A more conspicuous conformational dependence of the V_E^* curve in comparison with the V_E curve is in harmony with the explanation of the influence of effective charge of the CH_3 group which is much greater for the charges determining the V_E^* curve (0.141) than for the CNDO/2 *spd* charges determining the V_E curve (0.037). In case of the set of charges taken from [7], the intramolecular electrostatic interactions stabilize the TT conformation quite considerably in comparison with other positions so that this position is also much more stable in the resulting potential energy than the others. We have already mentioned that this prediction of the stabilization of the TT conformation has been confirmed neither by X-ray structural data on corresponding molecules nor by the MO calculations and is regarded as a shortcoming of the PFM method. Our results show that this shortcoming can be partially suppressed provided the CNDO/2 or *ab initio* charges are used for the calculation of the term V_E in PFM. In this case, the GG conformation appears as the most stable conformation of the phosphodiesteric unit according to PFM, but it is favoured very little with respect to TT and TG conformations. Notwithstanding, even in this case, the PFM method does not indicate a more distinct stabilization of the *gauche* conformation with respect to the *trans* conformation as documented in Fig. 2 by the V_{TOT} curve, representing the section of the conformational energy map for $\omega = 180°$. At the same time, it ensues from Fig. 2 that the CNDO/2 method in both kinds of parametrization predicts such stabilization. The preference of the *gauche* conformation was several times invoked on the basis of different quantum chemical calculations [13—16] and is also confirmed by the fact that almost exclusively the GG and GT conformations of phosphodiester have been observed experimentally. This phenomenon observed generally in the molecules containing an atom with lone electron pair in vicinity of polar bond is called anomeric or *gauche* effect and a discussion about its nature continues in literature [17—19]. The theoretical

treatment concerns mostly molecules containing two polar substituents of the type —F, —Cl, —OH, —OCH$_3$ on the central —CH$_2$— group [20—23]. It may be plausibly assumed that the conformation of the phosphodiesteric group exhibits an effect of similar character, but for the time being, the magnitude of the *gauche* stabilization remains vague in this case as well as its real origin.

Torsional potential with lone electron pairs included in the term V_E

The calculation of intramolecular electrostatic interactions in PFM on the basis of the MO charge distribution and eqn (2) represents a quite simplified procedure. One of the approximations in this procedure consists in the fact that the presence of lone electron pairs on electronegative atoms and their contribution to electrostatic interactions is not taken into account explicitly. As for the phosphodiesteric unit, it has been suggested that the dubious energetic preference of the TT conformation in PFM consists in omitting the mutual interaction of lone electron pairs of the esteric oxygen atoms [8] as well as their interaction with the vacant *d* orbital on the atom of phosphorus [9]. Therefore, we decided to investigate to what degree the inclusion of the interactions of lone electron pairs into the term V_E affects the character of torsional potential of the P—O bond in DMP$^-$.

By including the effect of lone electron pairs into the electrostatic interactions in molecule, we proceeded similarly as in the recent study of the structurally similar molecule of dimethoxymethane CH$_3$—O—CH$_2$—O—CH$_3$ [24]. The energy due to the interaction of lone electron pairs was calculated on the basis of the corresponding values of atomic hybridization dipoles. These dipoles arise as a consequence of nonuniform distribution of the electron charge localized on orbitals the centre of which is situated on a given atom. This anisotropy is especially marked if one of the hybrid orbitals is occupied by lone electron pair. The atomic hybridization dipole moments are usually obtained by the MO methods including CNDO/2 method we used on the basis of the Mullikan population analysis [12]. The vector sum of all atomic hybridization moments μ_h and of the dipole moment due to net charges localized on nuclei μ_q gives the total dipole moment of molecule μ. While both quantities μ and μ_q in case of the anion DMP$^-$ depend on the coordinate system used for calculation, the atomic dipole moments μ_h are invariant with respect to the coordinate system even in case of charged particles. In the CNDO/2 treatment with the *spd* basis set, we assumed in addition to the *sp* hybridization moment also the *pd* hybridization moment [25]. Thus, we obtained seven atomic hybridization moments (provided the *pd* hybridization on the atom of phosphorus was assumed, even eight atomic hybridization moments) for DMP$^-$ from the CNDO/2 calculations. Besides four μ_h from lone electron pairs on two O$_a$ and two O$_e$ oxygen

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atoms, there are two hybridization moments on carbon atoms and one (two for the *spd* basis) on the phosphorus atom included in this number. The hybridization dipole moments arise on C and P owing to the "nonideal" geometry of DMP⁻ (Fig. 1) in which the bond angles on C and P differ from the tetrahedral ones. While the values of μ_h for carbon atoms are relatively small (0.700×10^{-30} Cm according to CNDO/2 *spd*) in comparison with the dipoles on oxygen atoms O_e (3.769×10^{-30} Cm) and O_a (3.903×10^{-30} Cm), the dipoles on phosphorus show a comparable magnitude (2.201×10^{-30} Cm from the *sp* hybridization and 3.569×10^{-30} Cm from the *pd* hybridization). Fig. 3 schematically represents the orientation of all μ_h in DMP⁻.
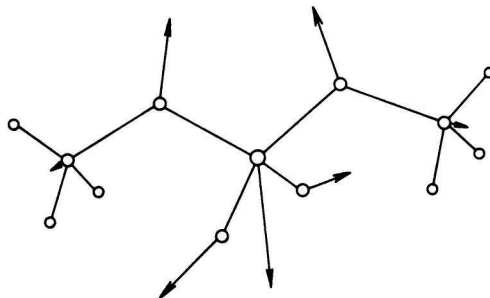

Fig. 3. Schematic representation of the magnitude and orientation of the atomic hybridization dipole moments of DMP⁻ in TT conformation from CNDO/2 *spd* calculation.

For the calculation of V_E according to eqn (*2*), we replaced each hybridization dipole in DMP⁻ (in case of hydrogen it equals zero) by a pair of point charges with opposite signs ($+Q_i$ and $-Q_i$). Thereby, we placed the negative charge into a distance determined by one-centred matrix element in the Slater basis of atomic orbitals of the type $\langle 2s/x/2p_x \rangle$ which ought to correspond to the centre of electron density of the hybrid orbital while the values of integrals were calculated according to [26]. The corresponding positive charge Q_i was placed on the nucleus and its value was added to the value of net charge on nucleus obtained from charge distribution. The magnitude of charges and their spatial distribution was so chosen that it represented the original atomic hybridization moment μ_h on a given atom. In this way, we expanded the original set of thirteen charges characterizing the charge distribution in DMP⁻ by seven (eight) additional charges by using the *sp* (*spd*) set of functions in CNDO/2. The values of additional charges Q in which the charges simulating the lone electron pairs on oxygen atoms are also included are listed in Table 2.

The calculation of the term V_E with the set of charges so expanded should also describe the spatial character of electrostatic interactions of the lone pairs on the oxygen atoms O_a and O_e. Their hybridization corresponds to the sp^2 type while the

Table 2

Values of additional charges Q calculated from the atomic hybridization dipole moments obtained by the CNDO/2 method (in e.u.)

	P	O_a	O_e	C
CNDO/2 *sp*	− 0.411*	− 0.791	− 0.725	− 0.035
CNDO/2 *spd*	− 0.208*	− 0.724	− 0.696	− 0.094
	− 0.532**			

* From *sp* hybridization.
** From *pd* hybridization.

second lone pair on oxygen atoms is of the *p* type and does not contribute to the value of μ_h or Q. A similar procedure simulating the influence of lone pairs by means of localized charges has been also applied in other cases, for instance in the study of the properties of water [27]. We used the expanded set of charges $(q + Q)$ for the calculation of the term V_E in DMP⁻, and together with the contributions V_{NB} and V_{TOR} for the construction of the map of conformational energies $V(\omega, \omega')$ as well.

If the $q + Q$ charges corresponding to the *sp* basis of CNDO/2 were used, the TG conformation was obtained as the most stable, the energy difference with respect to the TT conformation being 4.04 kJ mol⁻¹. For the $q + Q$ set corresponding to *spd* basis the most stable conformation is TT 6.66 kJ mol⁻¹ below the TG. In both cases, no minimum occurred in the region of the GG conformation. Therefore, the inclusion of additional charges does not result in suppressing the preference of the TT conformation, but, on the contrary, in its still greater stabilization. The situation is somewhat better if we have used only four additional charges belonging to the lone pairs on O_a and O_e and thirteen charges of the charge distribution for the calculation of the term V_E. In this case, the most stable is the TG conformation, then the GG conformation (less stable by 3.91 kJ mol⁻¹) and lastly the TT conformation (10.80 kJ mol⁻¹) follows. A simplified explanation of this order of stability can be seen in the mutual equilibrium of the interactions among lone electron pairs on O_a and O_e. The eclipsed position of lone pairs on O_e destabilizes the TT conformation. This interaction is replaced at the rotation of the methyl group by 120° to the TG position by the interaction of the lone pairs of the $O_a - O_e$ type the dipoles of which are also oriented approximately parallelly in this position. The rotation of another CH₃ group in the GG conformation of DMP⁻ results in the formation of two repulsively oriented pairs of dipoles of the type $O_e - O_a$. It follows from the comparison of the above-mentioned conformational energies that such double $O_e - O_a$ interaction is still preferable to the parallel position of the $O_e - O_e$ dipoles in the TT conformation.

The results of this part may be concluded by stating that even the inclusion of the charges simulating the presence of lone electron pairs in calculating the term V_E does not represent marked qualitative improvement of reliability of PFM as regards a prediction of the conformation of phosphodiesters. Although the preference of the TT conformation may be thus suppressed, the opposite order of the stability of the GG and TG conformations is obtained. Therefore, neither of the procedures used in this paper for calculating the term V_E results in a full removal of the disagreement between the results of PFM and X-ray structural results or quantum chemical calculations in the description of the torsional potential around the P—O bond.

Present limitations of PFM applications to biological phosphates

It is possible to find several factors which may contribute to the above--mentioned disagreement. One among them is the use of the Coulomb law for the determination of intramolecular electrostatic interaction on the basis of eqn (*2*) which is not theoretically well-founded. A more rigorous procedure ought to be based on segmental multipole expansion of electrostatic potential energy [28], but this approach has not yet been applied to the study of larger biological molecules because of its complexity.

The origin of the disagreement may however be also ascribed to interactions that are not amenable to the V_E term. For instance, the inclusion of the interactions of lone pairs by using hybridization dipoles involves only one part of their inter-actions. The stabilizing effects due to delocalization interactions of lone pairs (interactions through bonds) are by no means less important. In case of phos-phodiesteric unit, mainly the interactions between lone pairs of the p and sp type on O_e and the antibonding orbital of the P—O bond and PO_2^- group must be taken into consideration. From the CNDO/2 calculation it follows that the antibonding orbital of P—O is predominantly localized on the atom of phosphorus and its bonding interaction with the lone pair of the p type on O_e is maximum in the conformation $\omega = 90°$ and minimum in the *cis* and *trans* conformations (Fig. 4).

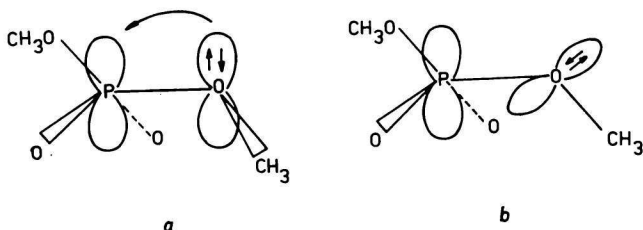*Fig. 4.* Delocalization of lone pair on oxygen into the vacant orbital on phosphorus when they are in the orthogonal conformation, $\omega = 90°$ (*a*) and the absence of interaction in the *trans* (or *cis*) conformation of DMP⁻ (*b*).

Similar interaction with the lone pair of the *sp* type on O_e exhibits the opposite character, *i.e.* it is maximum in the *cis* and *trans* position and minimum in the orthogonal conformation. The resulting dependence on conformation is given by the sum of both interactions of lone electron pairs of the *p* and *sp* type, which was demonstrated in full detail for dimethoxymethane [23]. Similar analysis could be possible even if the *d* orbitals on phosphorus were included. But we may assume that the interactions with O_e may not be much dependent on conformation because *d* orbitals exhibit much less directional character. This type of interactions of lone pairs through bonds is not included in PFM using eqn (*1*). Moreover, the delocalization interactions not only directly contribute to the form of torsional potential of the P—O bond but might modify it indirectly by producing rather remarkable change in the geometrical parameters of molecule. Therefore, a more accurate PFM treatment of biological phosphates evidently necessitates to include the contributions of $V(r)$ and $V(\Theta)$ expressing the potential energies of the deformations of bond lengths and bond angles, into eqn (*1*).

The preceding considerations concerning the effect of delocalization factors on torsional potential for a rotation around the P—O bond are also supported by the results ensuing from the expansion of rotational potential energy in a Fourier series

$$V(\omega) = \frac{V_1}{2}(1 - \cos \omega) + \frac{V_2}{2}(1 - \cos 2\omega) + \frac{V_3}{3}(1 - \cos 3\omega) \qquad (3)$$

while the second CH_3 rotor is fixed, *e.g.* in the position $\omega' = 180°$. For the rotation around one bond, the individual coefficients have been so interpreted [29, 30] that V_1 expresses the influence of dipole-dipole interaction, V_2 describes the delocalization interactions between lone pairs and adjacent antibonding orbitals and V_3 is the intrinsic barrier of threefold rotation around a single bond. Table 3 contains the

Table 3

Coefficients of the Fourier expansion of torsional potential for the rotation around the P—O bond in DMP^- (in $kJ\ mol^{-1}$)

	V_1	V_2	V_3	V'
PFM*	−0.29	−0.21	5.25	
CNDO/2 *sp*	11.97	−3.64	−0.60	
CNDO/2 *spd*	10.60	−3.93	−8.00	
*ab initio***	24.28	2.51	5.86	
*ab initio****	1.59	−10.80	−2.68	3.64

* Calculated with the use of the CNDO/2 *spd* set of net charges on atoms.
** Ref. [14].
*** Ref. [30].

coefficients of the Fourier expansion of torsional potential for the rotation around one P—O bond in DMP⁻, as found by different calculational methods. By comparing PFM with quantum chemical methods, we can observe that the different form of rotational potential predicted by these two kinds of methods is due mainly to different values of the V_1 and V_2 coefficients in the Fourier expansion. In other words, both types of methods in a different manner describe the intramolecular interactions the torsional dependence of which may be expressed by the functions $\cos \omega$ and $\cos 2\omega$. The neglect of the interaction of lone pairs through the bonds in DMP⁻ may be one of the factors contributing to underestimation of the V_2 coefficient in PFM. An additional introduction of the term with the V_2 coefficient from the Fourier expansion (3) into eqn (1) for the calculation of potential energy of molecule results in the required stabilization of the *gauche* conformation with respect to the *trans* conformation of DMP⁻ and in the order of conformations GG, GT, TT, as it has been earlier demonstrated by *Govil* [8]. However, we have shown that this procedure may not be interpreted as an inclusion of the mutual electrostatic interactions among lone pairs on the atoms of esteric oxygen O_e [8] (their torsional dependence contributes to V_1 term only) but as a consequence of the interaction between the lone pair on O_e and the central PO_2^- group.

Of course, the comparison of the coefficients of the Fourier expansion of rotational potential has the intrinsic limitation. Besides the reliability of the curve of total potential function, the form of function used for expansion also plays a role. Recently, *Hayes et al.* [30] modified function (3) by adding the term V_1' $\cos (\omega - \omega')$ and used it for the conformational analysis of H_3PO_4 and similar molecules. The results are summarized in Table 3, also pointing out the significance of the "anomeric" coefficient V_2 in conformational analysis of phosphates. The relatively high value of V_2 obtained by quantum chemical calculations indicates that the cause of anomeric effect in phosphates and acetals is of different character. For acetals, we demonstrated the electrostatic origin of the anomeric effect [24] since the inclusion of the electrostatic interaction of lone electron pairs according to eqn (2) resulted in the expected stabilization of *gauche* position and the coefficient V_2 in expansion (3) for acetal was relatively small. The results of this study on phosphodiesteric unit lead just to opposite conclusions in above-mentioned items, which could indicate a delocalization character of anomeric effect in phosphates [31].

Besides the absence of delocalization interactions and the coefficient V_2, there are also other factors in PFM which are worth of a more detailed examination. The origin of V_{TOR} in eqn (1) is not physically clear because there is not an unambiguous reason to assume the threefold barrier of torsion. In principle, its value can be calibrated by using quantum chemical calculations, analogously to the procedure described in [8]. However, the situation is rather complicated by the recent findings that the torsional potential of the P—O bond from the quantum

chemical calculations is considerably dependent on geometry of the phosphodiesteric unit [32—34]. The optimization of one degree of a freedom (O_e—P—O_e angle) only has shown [34] that, under certain conditions, even the TG conformation can be the most stable. The MO results with the optimized O_e—P—O_e angle in DMP$^-$ are no more so different from the PFM results as MO calculations with rigid geometry and indicate that the phosphodiesteric unit is much more flexible than it has been hitherto assumed on the basis of the MO results. The final answer could be given by the computations with the complete geometry optimization. On the other hand, our calculation of DMP$^-$ with the O_e—P—O_e angle changed to 98° has confirmed that the torsional potential in PFM is very little dependent on geometry of the molecule.

The most problematic factor in the comparison of the calculated and experimentally observed structures of phosphodiesteric unit is the effect of environment. The conformational properties computed for the isolated molecule can be quite different from the situation valid for the solution or crystalline state. The simulation of the medium effect in PFM on the change of electrostatic interactions and total potential energy of molecule by varying the value of dielectric constant (most frequently in the range 2—10) represents only rough approximation. The additional effects (presence of ions of a different kind, change in degree of an ionization, an ionic strength, pH, packing forces, *etc.*) should be successively included in the calculation in order to justify the validity of the comparison: calculation — experiment. The unambiguous absence of TT conformation in the phosphate unit can be questioned also from the experimental point of view since recently [35] the TT conformation was determined to be the most abundant in aqueous solution of DMP$^-$, contrary to observations discussed in [14—16].

As long as all mentioned effects and improvements are not included in the computational methods it is probably premature to reprobate one or another method and all should be developed. The MO methods will provide PFM with a set of intrinsically well-balanced and physically correct parameters which should be transferable to phosphodiesteric units in larger molecules. The PFM parametrization of this kind should make possible a quite reliable application of this method to the determination of structure of oligo- and polynucleotides.

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