

QUANTUM-CHEMICAL AND EMPIRICAL CALCULATIONS ON PHOSPHOLIPIDS VIII. THE ELECTROSTATIC POTENTIAL FROM ISOLATED MOLECULES UP TO LAYER SYSTEMS

G. PEINEL, H. FRISCHLEDER* and H. BINDER

Sektion Physik der Karl-Marx-Universität, WB Molekülphysik, DDR-7010 Leipzig, Linnéstrasse 5 (German Democratic Republic)

Received April 30th, 1982 accepted March 12th, 1983

Using 1-6-12 empirical functions with a solvent-averaged electrostatic contribution $qq_j/\epsilon(r_{ij}) \times r_{ij}$ and electrostatic potentials from CNDO-type wavefunctions, the development of specific interactions of ions visualized by the molecular electrostatic potential of PO_4 -group containing molecules was studied. Going from single molecules to monolayers made up of 37 head groups of phosphatidylcholine (PC) or phosphatidylethanolamine (PE) for quantum-chemical calculations, or of 23 head groups for empirical calculations we found decreasing potential minima. Only the inclusion of the screening effect of water, simulated by a distance dependent dielectric constant, $\epsilon(r)$, gives an explanation of stereospecific interactions of model membranes with ions. This finding can be compared with results of simulation calculations on water structure above a PE head group layer.

Keywords: molecular electrostatic potential; PO_4 -group; computer simulation; orientation correlation.

Introduction

Phospholipids are one of the main components of model and biomembranes. A great variety of experimental data from spectroscopic, scattering and calorimetric methods [1–3] can be completed by theoretical investigations on the molecular level. From a comparison of experimental and theoretical findings we get further information about the elementary steps at the level of atoms and molecules. The action of phospholipids is two-fold: in water they build supermolecular structures, e.g. lamellar systems, packed into a hexagonal lattice [4,5], and the polar head groups are in close contact with the environment and respond to slight alterations within the molecular neighbourhood. We can assume that the conformational behaviour of PE and PC head groups is known from experimental and theoretical investigations [6–10] but the study of interaction properties by theoretical methods is only little developed [11–15]. The aim of this paper is to present

*Deceased 30th April 1981.

results of a systematic study of the molecular electrostatic potentials (MEP) of PO₄-group containing molecules. Especially effects due to the superposition of the MEP of neighbouring molecules in layer models are studied, also including the influence of water. From our point of view water must be considered also in theoretical investigations because biological systems without water cannot exist.

Methods

The MEP is a widely used tool to describe molecular interaction properties of polar systems [16,17]. We concentrate our interest on this quantity, because the main components of membrane environments are ions, water and other polar molecules. The MEP $V(\vec{r})$ is defined by the charge distribution $\rho(\vec{r})$ and is a solution of the Poisson equation

$$\Delta V(\vec{r}) = -\frac{\rho(\vec{r})}{\epsilon_0} \quad (1)$$

Within the LCAO-MO framework the general solution can be given in terms of an atomic basis set $\{\chi_\mu\}$ [16]

$$V(\vec{r}) = \frac{1}{(4\pi\epsilon_0)} \left[\sum_{\alpha} \frac{Z_{\alpha}}{|\vec{r} - \vec{R}_{\alpha}|} - \sum_{\mu,\nu} P_{\mu\nu} \int \frac{\chi_{\mu}^*(\vec{r}')\chi_{\nu}(\vec{r}')}{|\vec{r} - \vec{r}'|} d^3r' \right] \quad (2)$$

The electric field

$$\vec{E}(\vec{r}) = -\nabla V(\vec{r}) \quad (3)$$

calculated by a simple difference expression [18] gives a clear picture of the strength and orientation of electrostatic forces on ions and dipoles. To limit the computational expense for MEP calculations we used simple valence basis CNDO-type wavefunctions [19]. The wavefunctions ψ_L for clusters of monolayer models are approximated by simple Hartree products of the wavefunctions of the isolated molecules ψ_i

$$\psi_L = \psi_1(1, \dots, 2n)\psi_2(2n+1, \dots, 4n) \dots \psi_K(\dots 2N) \quad (4)$$

This approximation is valid for layers made up of phospholipid head groups. Even in the region of dense packing the quasi-hexagonal structure is mainly caused by the van der Waals interaction of the hydrocarbon chains [20], while the polar head groups have only little influence, which is reflected by the phase transition temperature between 40°C and 60°C [21]. In a previous

paper we could show that the inclusion of mutual polarization alters the charge distribution in the head groups only slightly [22].

To avoid further computational effect for layer models we used 1-6-12 potentials in the parametrization of Momany et al. [23]

$$U(\vec{r}) = \sum_{\alpha} \left[332 \frac{q_l q_{\alpha}}{\epsilon(r_{\alpha}) r_{\alpha}} - \frac{c_{l\alpha}}{r_{\alpha}^6} + \frac{d_{l\alpha}}{r_{\alpha}^{12}} \right] \quad (5)$$

where \vec{r} now denotes the coordinates of a test charge (for our cases, a Na^+ ion) and $r_{\alpha} = |\vec{R}_{\alpha} - \vec{r}|$ is the distance between the test charge q_l and the atomic charge q_{α} . The q_{α} values were taken from CNDO-APSG calculations [22]. According to Ref. 23 the coefficients in Eqn. 5 were calculated.

The Lennard-Jones contribution in Eqn. 5 serves a stereospecific repulsive interaction only near the layer surface, while in all other cases the electrostatic potential in a point charge approximation will be calculated. Computer simulations on water structure above a PE headgroup layer were done using the well-known Metropolis algorithm [25] and the ST2-model of water [26]. For computational details see Ref. 27.

Geometries

For comparability with our previous calculations we used for the dimethylphosphate (DMP) anion the geometry data of Ref. 11, also DMP in *gauche-gauche* conformation. For the diethylphosphate (DEP) anion the data were taken from Ref. 28. Here we studied the *trans-trans* conformation. For the PE head group we used the bond length, bond and torsion angles from X-ray data [29]. For the PC moiety methyl groups were tetrahedrally added to the ethanolamine nitrogen with a bond length of 1.47 Å for N-C bonds. Hydrogen atoms were added with bond lengths 1.09 Å for C-H and 1.03 Å for N-H bonds.

Results and discussion

We divide our presentation into two main parts: firstly we discuss results of calculations on single phosphate group containing systems, and secondly we go on to layer models. In such a way we can distinguish between molecular and macromolecular effects in membrane electrostatics.

MEP of isolated phosphate groups

The most interesting region of phosphate groups in biomolecules are the anionic oxygen sites. Many calculations were done for the DMP anion by

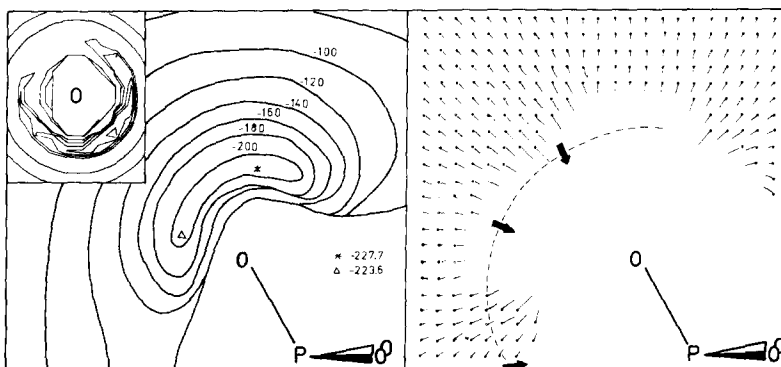


Fig. 1. The O-P-O plane of the DMP anion. (a) The MEP, in the top left corner the MEP in a plane perpendicular to the P-O-bond. (b) Electric field strength.

the Pullmans and their coworkers [30–33]. We use their results as a criterion for our calculations. In two points we find agreement: the values and positions of minima are due to the *ab initio* results* [30], and the MEP in a plane perpendicular to the P=O bond represents a three-fold symmetry (in the chemist's nomenclature representing lone pairs) (see Fig. 1a). These facts enable us to use semi-empirical CNDO wavefunctions also for molecules containing second row atoms. In Fig. 1b, we show the electric field patterns within the respective plane around the DMP anion. The field strength $\vec{E}(\vec{r})$ may serve to estimate the electrostatic interaction energy $E_{es}(\vec{r})$ in a hydration shell if \vec{r} is a position in a reference area. We constructed this area around the anionic oxygens with $r = 2.5 \text{ \AA}$, shorter than the usual H-bond length of about 2.85 \AA [34], knowing results of quantum chemical calculations on the hydration of the DMP anion [11,12,15]. In Fig. 1b we indicated the minimum regions for E_{es} from our electrostatic point of view. In the Table I we summarized these results and compared them with more extensive calculations. Clearly the orientation and interaction energy of water molecules follow the field strength, the main tendencies in the interaction energies can be learnt from electrostatics. Replacing the methyl groups in DMP by ethyl groups we obtain the DEP anion. The MEP around the anionic oxygens (see Fig. 2a) is very similar to that of the DMP anion. The minimum values differ only little and the positions of the minima are the same. The assumption that the phosphate group is a neighbourhood independent unit in biomolecules is underlined by results on an anionic PE headgroup model. In this case we 'deprotonated' the amino group and also studied the MEP in the region of the oxygens. Naturally, due to the molecular structure now the symmetry of the potential

*We cannot overrate the excellent agreement, because different geometries were used.

TABLE I

ELECTROSTATIC INTERACTION ENERGIES (IN kcal/mol) FOR VARIOUS HYDRATION SITES AROUND THE ANIONIC OXYGENS OF THE DMP ANION

The values in parentheses were obtained by dividing water into OH-bond dipoles of 1.58 D.

Hydration site ^a	This work	[11]	[12]	[15]
B ₁₃	11.1 (19.0)	25.95	27.1	18.5
E ₃₁	13.3 (11.3)	19.37	27.4 (24.3) ^b	19.0
L ^c	12.4 (10.6)	–	–	21.7

^aNotation following Ref. 12.

^bFor different orientations.

^cDefined in Ref. 15.

is disturbed, but the main tendency is reproduced (see Fig. 2b). We found minima of -220.8 kcal/mol. We can estimate from our discussion about monohydration in the region of the DMP⁻ oxygens that the situation for all three anionic systems is comparable. The orientation of attached water-molecules follows the electric field orientation either as a whole (this means, the dipole moment) or for single water O–H bonds. So we can explain the existence of bridged (B), open (E) and linear (L) monohydrate structures*. In the bridged configuration the two O–H bond dipoles are oriented partly independently, while in E- and L-configurations only one bond dipole contributes to the total interaction energy. So the interaction energies for E- and L-structures are similar, while B-structure energy is different. We can briefly summarize the results on zwitterionic PE- and PC-head groups. Because of the partly anionic character of phosphate group oxygens the potential minima are lower than other carboxyl minima [35]. But also here the great similarity is evident, as can be seen in Fig. 2c,d. Since it is only influenced by steric effects of neighbouring atomic groups in all systems, the PO₄ unit acts as preferred interaction site for cations and water. This action only reaches a first hydration shell and this information can be utilized for discussions of effects on complex biological membranes. B. Pullman showed that the MEP of DMP⁻ is screened by water molecules in a first assumed hydration shell. In the region of interest the MEP minimum increases by about 100 kcal/mol and the resulting potential at the outer sphere is more similar to the water one than that of DMP⁻ (see Fig. 10 of Ref. 13). How the phosphate group plays this special role in greater assemblies will be considered in the next section.

*Notation following Pullman et al. [12] Fig. 2, and Gay and Vanderkooi [15] Fig. 2.

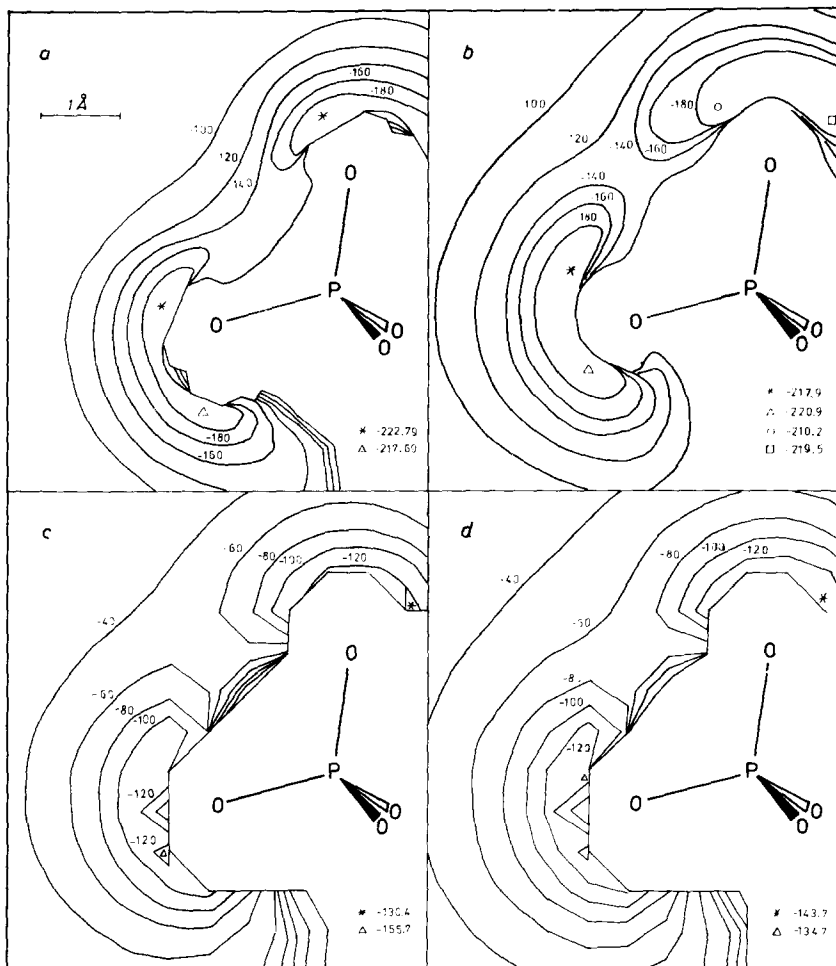


Fig. 2. MEP in the O-P-O plane of anionic oxygens of: (a) DEP anion; (b) PE anion; (c) PE zwitterion; (d) PC zwitterion (values in kcal/mol).

MEP of phospholipids in monolayers

The lattice vectors of the PE- and PC-headgroup arrangements in a monolayer were taken to be

$$\begin{aligned} \vec{a}_1 &= (7.77, 0.) & \text{for PE [29,36] and} & \vec{a}_1 = (8.56, 0.) \\ \vec{a}_2 &= (3.85, 5.) & & \vec{a}_2 = (4.28, 5.38) \end{aligned}$$

for PC [37]. The unit cell contains one molecule. In this arrangement, one of the possibilities from experimental data, the head group dipole moments are nearly linearly oriented. Zakrzewska and Pullman [38] showed that the main feature of the monolayer MEP of lipid headgroups is not significantly altered by an opposite layer forming a bilayer. We performed two types of calculations, quantum-chemical calculations for clusters of 37 headgroups in a quasi-hexagonal arrangement using the lattice parameters given above. We started the investigation for the smallest possible cluster made up of one central molecule and the six next nearest neighbours. Even for 37 headgroups we could not reach convergence for the minimum values because of the high polarity of the molecules. For economic reasons we finished the calculation at this number. That means that complex ordered structures need a large number of elements within the cluster to model the real interface properties. In the 37-molecule cluster the central group has only three neighbours in each direction, which is too little to obtain significant values. As we also found for a one-dimensional surface model of LiH at least six neighbours in each direction are necessary to obtain bulk-like MEP minima (Peinel, unpublished data). It seems to be useful to go over to approximate methods as usual in the Pullmans' group [39]. Besides the numerical inaccuracy of the MEP values two main features occur:

- (1) in all cases the minimum region is near to the phosphate anionic oxygens;
- (2) the largest gradient of MEP was found between the phosphate and nitrogen groups.

In a highly simplified manner we included the influence of hydration layers on the resulting electrostatic potential. Instead of the usual fixed dielectric constant in Eqn. 5 we included a function $\epsilon(r)$, which represents the behaviour of ϵ between 1 and the bulk value 80. The respective curve is given in Refs. 14 and 27. For such a modified electrostatic potential, convergence was reached for a cluster size of 23 molecules [14]. We included a first tightly bound water molecule near to the phosphate group. The resulting MEP of the layer [14,27] shows that the consideration of microscopic and macroscopic water effects gives a realistic picture of the interaction properties of head group layers. The main difference was found for PC. While from quantum-chemical calculations we found low minima around the phosphate group, only slightly higher than for PE, from empirical calculations we obtained only repulsive regions for the interaction with anions. The latter results are in agreement with experimental findings, only for PE layers under special conditions a stereospecific interaction with anions could be detected, while PC lamellar systems cannot interact with ions [40,41].

What does MEP teach us?

Water structure above a PE headgroup layer

In a very restricted computer simulation of the structure of 16 water molecules per one PE headgroup in a layer in a fixed X-ray conformation and configuration we found a clear influence of the layer MEP. In Fig. 3 we present the hypersurface of the water-layer interaction for a fixed layer-oxygen distance [27]. Following the electric field strength of the layer the minimum region was found between the phosphate and ammonium group. In principle the addition of further water molecules does not alter this situation. If we study the orientation correlation function

$$G(Z) = \frac{\left\langle \vec{\mu}_{HG} \left(\sum_{Z_i \leq Z} \vec{\mu}_i(\vec{r}) \right) \right\rangle}{\mu_{HG} \mu_i} \quad (6)$$

of the water dipoles $\vec{\mu}_i$ with respect to the head group dipole moment $\vec{\mu}_{HG}$ up to a fixed distance Z normal to the plane we can see that the first four water molecules are influenced by the field of the real charge distribution, while more distant water follows the superimposed field of head group point

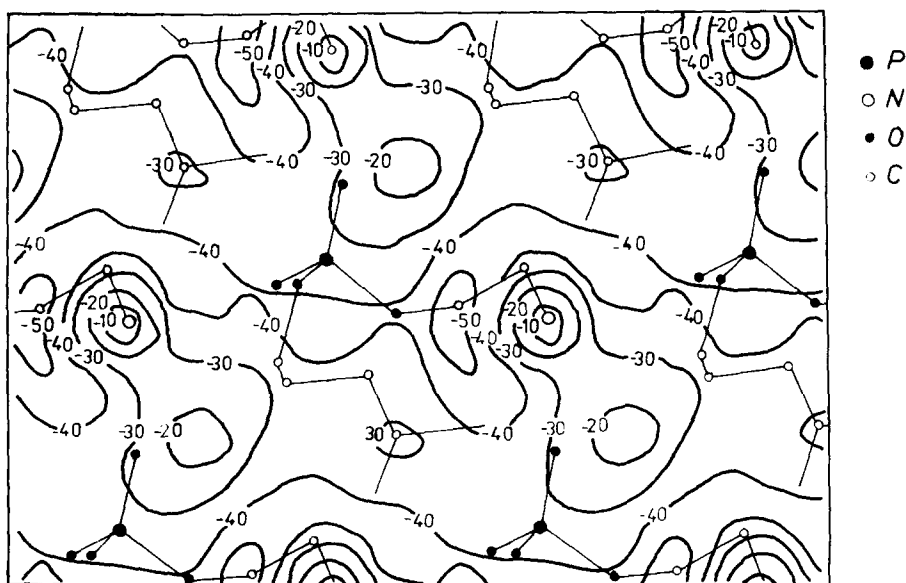


Fig. 3. The energy profile of the interaction of one water molecule above a PE head group layer. At each point the water orientation is optimized to reach the local minimum.

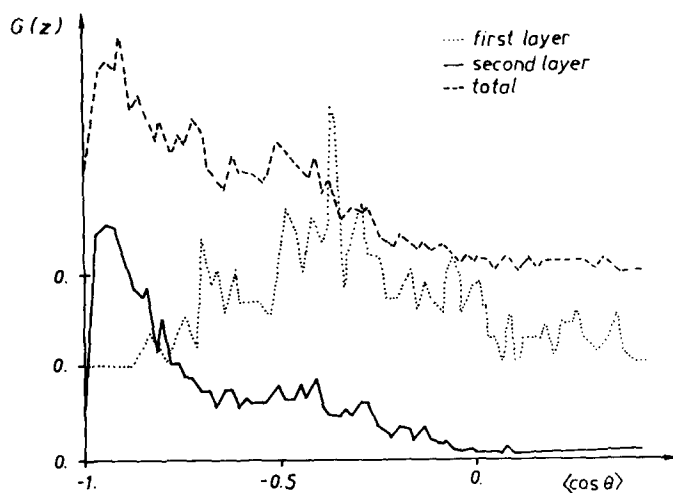


Fig. 4. Orientation correlation function $G(Z)$ for the first layer (....), the second layer (—), and all (----) of water molecules.

dipoles. The maximum of $G(Z)$ for the tightly bound waters at $\cos \theta = -0.4$ reflects an approximately perpendicular orientation with respect to $\vec{\mu}_{HG}$ near the phosphate and nitrogen groups (cf. Fig. 4). The summarized function $G(Z)$, as shown with a broken line in Fig. 4, indicates the tendency of water molecules to compensate the in-plane dipole component of the head groups. The component distribution (X , Y within the plane, Z normal to the plane; in D units) is as follows:

Contribution of	X	Y	Z	Total
Head group	19.51	6.98	-2.06	20.82
Water	-10.87	-6.77	-0.65	12.82
Total	8.64	0.21	-2.71	9.06

The obtained structural information about water near the lipid-water interface can be clearly interpreted in terms of the MEP of the polar layer. As also discussed for the supermolecule hexahydrate of the DMP anion [13] the electric field acts directly only on the first hydration layer. In more distant regions the field and the MEP are drastically reduced. On the other hand the results of computer simulations justify the use of the empirical coulomb potential reduced by a distance dependent dielectric constant. Further progress can be expected from the combination of exact calculations with physically based approximate theories.

Conclusions

We can summarize our results as follows:

(i) Semi-empirical wavefunctions can be used even for MEP calculations on complicated systems containing second-row atoms. The numerical agreement with extended ab initio calculations is very good.

(ii) On the basis of the MEP of single phosphate group containing molecules we found that the phosphate group has a special character under all conditions. Independent of the molecular neighbourhood, the anionic oxygens interact strongly with anions and water.

(iii) The MEP can be used to interpret complicated systems, such as layer-water complexes, to discuss elementary steps and interaction properties.

Acknowledgements

Helpful discussions with C. Werge and K. Gawrisch are gratefully acknowledged. We wish to thank Mrs. U. Krüger for help in organizing the calculations and drawing the pictures. The generous provision of computer time at the university EC 1040 made the calculations possible.

References

- 1 E. Oldfield, M. Meadows and M. Glaser, *J. Biol. Chem.*, 251 (1976) 6147.
- 2 C.L. Khetrapol, A.C. Kunwar, A.S. Tracey and P. Diehl, in: P. Diehl, E. Fluck and R. Kosfeld (Eds.), *NMR Basic Principles and Progress*, Vol. 9, Springer-Verlag, Berlin, Heidelberg, New York, 1975.
- 3 M.K. Jain and N.M. Wu, *J. Membr. Biol.*, 34 (1977) 157.
- 4 D.M. Engelman, *J. Mol. Biol.*, 47 (1970) 115.
- 5 K. Fontell, *Mol. Cryst. Liq. Cryst.*, 63 (1981) 59.
- 6 S.P. Gupta and G. Govil, *FEBS Lett.*, 27 (1972) 68.
- 7 B. Pullman and A. Saran, *Int. J. Quant. Chem.*, S2 (1975) 71.
- 8 R.H. Pearson and I. Pascher, *Nature*, 281 (1979) 499 and Refs. therein.
- 9 H. Frischleder and R. Lochmann, *Int. J. Quant. Chem.*, 16 (1979) 203.
- 10 H. Frischleder, *Chem. Phys. Lipids*, 27 (1980) 83.
- 11 H. Frischleder, S. Gleichmann and R. Krahl, *Chem. Phys. Lipids*, 19 (1977) 144.
- 12 B. Pullman, A. Pullman, H. Berthod and N. Gresh, *Theor. Chim. Acta*, 40 (1975) 93.
- 13 B. Pullman, *Frontiers in Physicochemical Biology*, Academic Press, New York, 1978, p. 143.
- 14 H. Frischleder and G. Peinel, *Chem. Phys. Lipids*, 27 (1980) 71.
- 15 R. Gay and G. Vanderkooi, *J. Chem. Phys.*, 75 (1981) 2281.
- 16 E. Scrocco and J. Tomasi, *Adv. Quantum Chem.*, 11 (1978) 115.
- 17 C. Petrongolo, *Gazz. Chim. Ital.*, 108 (1978) 445.
- 18 G. Peinel, H. Frischleder and F. Birnstock, *Theor. Chim. Acta*, 57 (1980) 245.
- 19 J.A. Pople and D.L. Beveridge, *Approximate Molecular Orbital Theory*, McGraw-Hill, New York, 1970.

- 20 G. Büldt, H.U. Gally, J. Seelig and G. Zaccari, *J. Mol. Biol.*, 134 (1979) 673.
- 21 K. Gawrisch, K. Arnold, H.-J. Rüger, P. Kertscher and P. Nuhn, *Chem. Phys. Lipids*, 20 (1977) 285.
- 22 G. Peinel, *Chem. Phys. Lipids*, 14 (1975) 268.
- 23 F.A. Momany, R.F. McGuire, A.W. Burgess and H.A. Scheraga, *J. Phys. Chem.*, 79 (1975) 2361.
- 24 L. Pauling, *Natur der chemischen Bindung*, Verlag Chemie, Weinheim, 1964, p. 475.
- 25 M. Metropolis, A.W. Rosenbluth, M.N. Rosenbluth, A.H. Teller and E. Teller, *J. Chem. Phys.*, 21 (1953) 1087.
- 26 A. Rahman and F.H. Stillinger, *J. Chem. Phys.*, 55 (1971) 333.
- 27 H. Frischleder and G. Peinel, *Chem. Phys. Lipids*, 30 (1982) 121.
- 28 G. Corongiu and E. Clementi, *Gazz. Chim. Ital.*, 108 (1978) 687.
- 29 M. Elder, P. Hitchcock, R. Mason and G.G. Shipley, *Proc. R. Soc. London Ser. A*, 354 (1977) 157.
- 30 A. Pullman and H. Berthod, *Chem. Phys. Lett.*, 32 (1975) 233.
- 31 D. Perahia, A. Pullman and H. Berthod, *Theor. Chim. Acta*, 40 (1975) 47.
- 32 A. Pullman, in: B. Pullman (Ed.), *Environmental Effects on Molecular Structure and Properties*, D. Reidel Publ. Co., Dordrecht, 1976, p. 1.
- 33 B. Pullman and A. Pullman, in: B. Pullman and N. Goldblum (Eds.), *Metal-Ligand Interactions in Organic Chemistry and Biochemistry*, D. Reidel Publ. Comp., Dordrecht, 1977, p. 65.
- 34 A.H. Narten and H.A. Levy, in: F. Franks (Ed.), *Water – A Comprehensive Treatise*, Vol. 1, Plenum Press, New York, London, 1972.
- 35 R. Lochmann, G. Peinel and T. Weller, *Int. J. Quantum Chem.*, 20 (1981) 1097.
- 36 P.B. Hitchcock, R. Mason, K.M. Thomas and G.G. Shipley, *Proc. Natl. Acad. Sci. U.S.A.*, 71 (1974) 3036.
- 37 M. Diehl, Thesis, Halle, 1980.
- 38 K. Zakrzewska and B. Pullman, *FEBS Lett.*, 131 (1981) 77.
- 39 A. Goldblum, D. Perahia and A. Pullman, *Int. J. Quantum Chem.*, 15 (1979) 121.
- 40 J.A. Magnuson and N.S. Magnuson, *Ann. N.Y. Acad. Sci.*, 204 (1973) 297.
- 41 K. Arnold, R. Pausch, J. Frenzel and E. Winkler, *Stud. Biophys.*, 51 (1975) 81.