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FTIR-spectroscopic characterization of phosphocholine-headgroup model compounds

W. Pohle[a,*], D.R. Gauger[a], H. Fritzsche[a], B. Rattay[b], C. Selle[c], H. Binder[c], H. Böhlig[d]

[a]*Institute of Molecular Biology, Friedrich-Schiller University Jena, Winzerlaer str. 10, D-07745 Jena, Germany*
[b]*Institute of Pharmaceutical Chemistry, Martin-Luther University, Halle, Germany*
[c]*Institute of Experimental Physics I, University of Leipzig, Leipzig, Germany*
[d]*Institute of Physical Chemistry, University of Leipzig, Leipzig, Germany*

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Abstract

The polar headgroups are an important molecular subregion of phospholipids since they mediate a substantial part of the interactions emerging with other constituents of biological membranes, be it inherent macromolecules (proteins) or water as forming the natural environment. FTIR spectroscopy is well proven for characterizing aspects of weak interactions, above all hydrogen bonding. We have used this method to study solid deposits of a number of selected lipid models, such as choline, acetylcholine and methylphosphocholine (MePC), and compared them with the common phospholipid dimyristoylglycero-phosphocholine (DMPC), at different degrees of hydration, which were varied via relative humidity (RH).

At low RHs, only MePC and DMPC take up considerable amounts of water, thus elucidating the essential role of phosphate groups in the first stages of lipid hydration. The progress of PC-headgroup hydration can be sensitively monitored by the wavenumber decrease of the band owing to antisymmetric PO_2^- stretching vibration. Concomitant variations of the spectral parameters of νCH bands of MePC reveal that conformational changes may simultaneously occur in the headgroup. Surprisingly to us, the model compounds display qualitative differences in the appearance of their νCH bands, which are most probably a result of substituent effects.

Keywords: Phospholipids; Choline; FTIR spectroscopy; Hydration; Conformation

1. Introduction

Lipids represent an important class of biologically essential molecules. Their most meaningful property can be seen in amphiphilicity, i.e. the coexistence of polar and apolar regions in one and the same molecule, enabling lipids to form supramolecular assemblies and aggregations. The polar domain of phospholipids can be assumed to play a major role in the interplay of the weak forces relevant in molecular cell biology since it both bears charges which may exert Coulombic forces against the environment and provides sites for the specific binding from water molecules as the main constituents of the natural medium or, alternatively, other membrane components (proteins, sterols) and membrane-active compounds (peptides, drugs). Among phospholipids as the class of lipids most abundant in eucaryotic organisms, phosphatidylcholines (PCs) are especially widespread and generally enriched in the outer leaflet

* Corresponding author. Tel.: +49-3641-657566; fax: +49-3641-657520.
E-mail address: wapo@molebio.uni-jena.de (W. Pohle).

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of the bilayers constituting the biological membranes [1] presumably in order to improve the barrier function against hydrophobic matter.

FTIR spectroscopy is one of the most powerful and frequently used methods to characterize lipid systems on a submolecular level [2–4]. However, previous IR-spectroscopic studies were devoted mostly to the apolar region [2,3]. In a series of recent papers, efforts have been made to focus the potential of IR spectroscopy onto the headgroup region of phospholipids mainly by considering the spectral implications owing to hydration-driven isothermal (lyotropic) phase transitions [5–8]. Conformational changes have been suggested to occur in PC-headgroups on the basis of spectroscopic data obtained by NMR [9,10] as well as IR spectroscopy [5–8,11]. Relevant interpretations of previous IR-spectroscopic results referred mostly to features of the phosphate moiety [4–7] or to the ammonium residue [5,8,11]. Bands due to the two methylene groups of the PC headgroups which might be also influenced by relevant conformational changes can hardly be observed in the IR-spectra region of usual PCs since they are overlapped by the spectroscopic features arising from the large majority of chain methylene groups. To avoid this disadvantageous situation, we have recorded IR spectra of the basic PC model compound methylphosphocholine (MePC). Moreover, choline and acetylcholine as close relatives with the same $-(CH_2)_2-N^+(CH_3)_3$ moiety were investigated correspondingly. It should be mentioned that all these three compounds are not only of academic interest as suitable PC models, but each of them also has some biological meaning. MePC naturally occurs in the eggs of sea urchins [12], acetylcholine is a neurotransmitter, and choline is an important metabolite in biochemical pathways. The latter two compounds are also interesting from the pharmacological point of view. To model the phosphate moiety in biomolecules (DNA, phospholipids), mainly dimethylphosphate salts have been studied by IR spectroscopy [13,14] and in relevant theoretical calculations [14–17] up to now.

2. Experimental

2.1. Materials

Choline bromide and acetylcholine iodide were purchased from Sigma Co. (Munich, Germany), DMPC was from Bachem (Heidelberg, Germany). All these substances were used without further purification. MePC, being unavailable commercially, had to be synthesized. Among the different protocols given in the literature, the procedure according to Geilen et al. [18] was selected as the most suitable one. The details of MePC synthesis will be given elsewhere (Rattay et al., in preparation). Briefly, tetrabutylammoniumphosphorylcholine was reacted with methyliodide under appropriate conditions, and the resulting MePC was refined in several steps and analyzed by different methods (elemental analysis, mass spectrometry, NMR spectroscopy).

2.2. FTIR-spectroscopic measurements

Samples of the substrates were obtained as solid deposits by placing the material on IR-transparent ZnSe windows either from aqueous (choline, acetylcholine, MePC) or chloroformic solutions (DMPC) and measured in situ. Infrared spectra were recorded by means of an IFS-66 FTIR spectrometer from Bruker (Karlsruhe, Germany) in the DTGS-detection mode using different accumulation rates between 2 and 32 scans at a resolution of 2 cm^{-1} and a zero-filling factor of 2. Samples were investigated at different values of the surrounding relative humidity (RH) between 0 and 100% either isopiestically or in non-stationary hydration or dehydration scans. Data processing was done by the OPUS software package (Bruker) with a wavenumber accuracy of 0.1 cm^{-1}. All other experimental details were described previously [7]. Deviating from the algorithm used there [7], A_{OH} for MePC was determined in the following way: the total absorbance $(A_{OHCH})_0$ obtained by integration between 2500 and 3750 cm^{-1} of the dry sample was subtracted from the A_{OHCH} value resulting at either degree of hydration, and the difference was then divided by $(A_{OHCH})_0$.

3. Results and discussion

The overall IR spectra of choline, acetylcholine, MePC and DMPC are shown in Fig. 1. Some of the due results are discussed in terms of different spectral ranges, without regarding νCO and νCC bands.

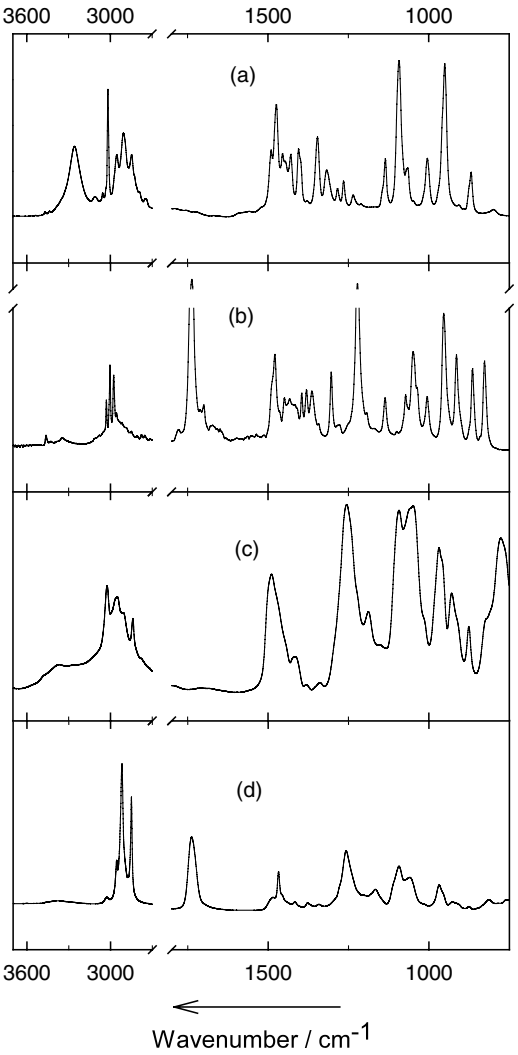

Fig. 1. FTIR spectra of: (a) choline, (b) acetylcholine, (c) methylphosphocholine, and (d) dimyristoylglycerophosphocholine measured as water-depleted deposits in an environment of 0% RH.

3.1. Region of νOH bands

In the νOH range of dry compounds, only one striking absorption is observed; this is a band near 3270 cm^{-1} arising in the case of choline, which can be assigned to OH stretching vibration. Whereas acetylcholine does not exhibit remarkable features in this region, the small broad bands with maxima around 3370 and 3380 cm^{-1} observed in MePC and DMPC, respectively, belong most probably to the so-called residual water, which is usually retained in PCs under conditions of 0% RH [7]. Upon progressive hydration, choline and its derivatives display significant differences in their capacity to imbibe water. First of all, any of the studied substances can be considered hygroscopic; this is simply revealed by the fact that they take up water to an extent that the deposits eventually slip down the support. At low RHs ($\leq$23%), only MePC and DMPC are able to bind substantial amounts of water in contrast to choline and acetylcholine. This as well as the difficulty to completely dry MePC and DMPC illuminate the particular role played by phosphate groups especially in the first steps of phospholipid hydration (cf. Refs. [7,19], and papers cited therein). Besides, a strong water uptake starts for choline much earlier (at 44% RH) than for acetylcholine (at 86% RH). The "hygroscopicity" of DMPC refers largely to the low-hydration range and is manifested by the existence of the residual water (see above).

3.2. Region of νCH bands

The wavenumbers of the peak maxima of IR absorption bands arising in the 3050–2800 cm^{-1} region show clear differences for the PC-model compounds. The only consistently occurring band is that situated around 3030 cm^{-1}, which can be attributed to the νCH mode of the nitrogen-bound methyl groups (this band will be discussed for MePC in the next section, see Fig. 2b). Further significant νCH bands emerge at about 2952, 2904 and 2845 cm^{-1} for choline, at ~3003, 2976 and 2956 in acetylcholine, around 2950, 2910 and 2840 cm^{-1} for MePC and at 2920 and 2850 cm^{-1} for DMPC (as for other long-chain PCs, too [2–8]). While the wavenumbers of νCH bands, $\tilde{\nu}$CH, are similar for choline and MePC, those of acetylcholine deviate dramatically, and we note here especially the absence of any significant absorption in the 2950–2800 cm^{-1} region. Altogether, these data indicate that $\tilde{\nu}$CH of methylene groups can be very strongly influenced by their relevant substituents. We are currently trying to explore these phenomena in more detail by the use of theoretical methods. The hydration-dependent behaviour of the MePC band at 2840 cm^{-1} is explored in Section 3.3.

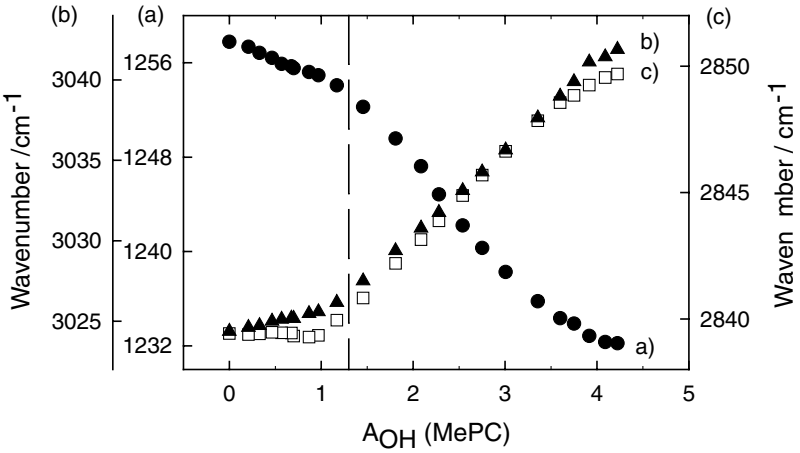

Fig. 2. Wavenumbers of the bands owing to antisymmetric PO_2^- stretch (a) and the C–H stretches near 3035 (b) and 2840 cm^{-1} (c) of MePC depicted versus A_{OH}, the degree of hydration, which was evaluated as explained in Section 2.2. The vertical line marks discontinuities occurring in the courses of all three graphs.

3.3. PC-headgroup bands

From the several IR modes representing the phosphate moiety, the band arising from the antisymmetric stretching vibration, $\nu_aPO_2^-$, is particularly sensitive against structural changes concerning relevant biomolecules, as phospholipids or DNA (see Refs. [4,7,8,19], and papers cited therein). Hydrating PCs from 0 to 100% RH, the wavenumber of this band, $\tilde{\nu}_aPO_2^-$, decreases usually from ~1260 to ~1230 cm^{-1}, i.e. by about 30 cm^{-1}, as demonstrated in several cases [7], and especially also for DMPC [20]. Phosphate groups are accepted generally to act as the primary binding sites for water in biomolecules. Supported by the results of corresponding quantum-chemical calculations, the strong hydration-induced decline of $\tilde{\nu}_aPO_2^-$ is in accord with the assumption that water molecules are hydrogen-bonded to the free oxygens of the phosphate group [21]. However, this does not exclude the fact that $\tilde{\nu}_aPO_2^-$ is additionally affected by changes in the PC conformation, which may occur simultaneously.

MePC shows a $\tilde{\nu}_aPO_2^-$ shift nearly as large as that of PCs (cf. Fig. 2a) suggesting strong binding of water to the phosphate also in this case, in contrast with the conclusions drawn in a previous IR-spectroscopic study of MePC [22]. One striking difference in the characteristics of the graphs of common PCs (cf. e.g. Fig. 7 in Ref. [7]) and MePC is that $\tilde{\nu}_aPO_2^-$ declines exponentially in the former case [7,20] and decreases relatively weakly in the initial hydration steps in the latter case. Supposedly, this is related to the fact that MePC forms a stable hemihydrate in the first stage of hydration as reported recently [22], and the binding of more water is somewhat retarded. Significant discontinuities at the same water content confirming this interpretation appear also in the A_{OH} dependences of other spectral parameters of MePC (cf. curves b and c in Fig. 2, where the locus of discontinuities in graphs (a–c) is marked by a broken vertical line; further corresponding data are not shown).

The other two graphs in Fig. 2 display how the wavenumbers of two C–H stretching-vibration bands around 3035 (b) and 2845 cm^{-1} (c) depend on hydration. While the former band can be assigned easily to the C–H stretch of N-bound methyl groups (see above), the assignment of the latter band is disputable. Interestingly, both these parameters behave like reversed images referred to $\tilde{\nu}_aPO_2^-$, thus suggesting that all these magnitudes are correlated with each other. Since direct water binding to C–H unities is rather unlikely to occur, the course of the $\tilde{\nu}$CH graphs in Fig. 2b and c can be — in contrast with the graph according to Fig. 2a — unambiguously interpreted as reflecting the occurrence of conformational change(s) in MePC accompanying the process of water binding to phosphate (we consider highly improbable that the

decrease of $\tilde{\nu}_a PO_2^-$ reflects only headgroup-conformation changes of MePC). Similar conformational transitions triggered by hydration might proceed in the headgroups of genuine PCs, too, but to detect them would demand the use of isotopically labeled compounds.

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References

[1] M. Langner, K. Kubica, Chem. Phys. Lipids 101 (1999) 3.
[2] H.L. Casal, H.H. Mantsch, Biochim. Biophys. Acta 779 (1984) 381.
[3] R.N.A.H. Lewis, R.N. McElhaney, in: H.H. Mantsch, D. Chapman (Eds.), Infrared Spectroscopy of Biomolecules, Wiley-Liss, New York, 1996, p. 159.
[4] W. Hübner, A. Blume, Chem. Phys. Lipids 96 (1998) 99.
[5] W. Pohle, C. Selle, Chem. Phys. Lipids 82 (1996) 191.
[6] H. Binder, A. Anikin, B. Kohlstrunk, G. Klose, J. Phys. Chem. 101 (1997) 6618.
[7] W. Pohle, C. Selle, H. Fritzsche, H. Binder, Biospectroscopy 4 (1998) 267.
[8] H. Binder, W. Pohle, J. Phys. Chem. B (2000) (December).
[9] B. Bechinger, J. Seelig, Chem. Phys. Lipids 58 (1991) 1.
[10] A.S. Ulrich, A. Watts, Biophys. J. 66 (1994) 1441.
[11] D. Hadzi, J. Grdadolnik, J. Chim. Phys. 90 (1993) 1771.
[12] B.S. Szwergold, F. Kappler, L.H. Cohen, D. Nanavati, T.R. Brown, Biochem. Biophys. Res. Commun. 172 (1990) 855.
[13] T. Shimanouchi, M. Tsuboi, Y. Kyogoku, Adv. Chem. Phys. 7 (1964) 435.
[14] Y. Guan, C.J. Wurrey, G.J. Thomas, Biophys. J. 66 (1994) 225.
[15] A. Pullman, H. Berthod, N. Gresh, Chem. Phys. Lett 33 (1985) 11.
[16] B. Jayaram, M. Mezei, D.L. Beveridge, J. Am. Chem. Soc. 110 (1988) 1691.
[17] D. Hadzi, M. Hodoscek, J. Grdadolnik, F. Avbelj, J. Mol. Struct. 266 (1992) 9.
[18] C.C. Geilen, A. Samson, T. Wieder, H. Wild, W. Reutter, J. Labelled Compd. Radiopharm. 31 (1992) 1072.
[19] W. Pohle, J. Mol. Struct. 219 (1990) 281.
[20] D.R. Gauger, W. Pohle, C. Selle, H. Fritzsche, J. Mol. Struct. 561–562 (2001) (in press).
[21] W. Pohle, M. Bohl, H. Böhlig, J. Mol. Struct. 242 (1991) 333–342.
[22] A.C. Akin, K.M. Harmon, J. Mol. Struct. 319 (1994) 55.