Biochimica et Biophysica Acta, 470 (1977) 492—496

BBA Report

BBA 71314

COMMENTS ON THE QUANTITATIVE INTERPRETATION OF BIOMEMBRANE STRUCTURE BY RAMAN SPECTROSCOPY

BÉLA KARVALY and EVGENIJA LOSHCHILOVA
Institute of Biophysics, Biological Research Center, Hungarian Academy of Sciences, H-6701 Szeged, P.O.B. 521 (Hungary)
(Received August 8th, 1977)

Summary

The possibility of quantitation of information obtained from laser Raman spectra of aqueous lipid dispersions is discussed. It is shown that the all-*trans* chain order parameter S_T introduced by Gaber and Peticolas ((1977) Biochim. Biophys. Acta 465, 260) for the characterization of biomembrane structure is of restricted applicability. This order parameter may give adequate information if polar head groups are not affected at all by the interaction resulting in *trans-gauche* isomerization. To demonstrate this, data on the effects of mono- and divalent ions on the all-*trans* chain order parameter are given. The lateral order parameter proved to be suitable for quantitative studies even in the case of ion-head group interaction.

In a recent comprehensive analysis of the laser Raman spectra of biomembranes and membrane models Gaber and Peticolas [1] suggested a method and several considerations for quantitative characterization of the lipid structure in membranes. For this purpose, appropriate structure-sensitive order parameters were introduced and applied to show the thermally-induced structural changes in vesicles and aqueous lamellar dispersions of dipalmitoyl phosphatidylcholine. The convincing agreement between the data obtained with laser Raman and calorimetric techniques may give the impression that the suggested procedure of standardization and quantitation of the Raman data is of general validity. The sensitivity of the membrane structure to the ionic composition of the aqueous solution is particularly interesting physiologically, while the thermal effects are usually of secondary importance. For this reason alone we examine the applicability of the order parameters suggested by Gaber and Peticolas to ion-induced structural alterations of model membranes. In this short communication some experimental data and theoretical arguments will be presented which refine and reconsider Gaber and Peticolas' suggestion, in order to achieve a more correct molecular interpretation of the laser Raman spectra of lipids in either aqueous dispersions or biomembranes if ion-membrane interactions take place.

For quantitative characterization of the bilayer structure of different lipid-water systems, Gaber and Peticolas defined two order parameters, one for the all-*trans* structure of hydrocarbon chains, S_T,

$$S_T = \frac{\left(\frac{I_T}{I_{\text{ref}}}\right)_{\text{sample}}}{\left(\frac{I_T}{I_{\text{ref}}}\right)_{\text{crystalline lipid}}} \tag{1}$$

and another one for the lateral packing density of hydrocarbon chains, S_L,

$$S_L = \frac{(I_{CH_2})_{\text{sample}} - 0.7}{1.5} \tag{2}$$

I_T is the intensity of the Raman line assigned to the A_g vibrational mode of the all-*trans* chain conformation, which is located at about 1130 cm^{-1}. I_{ref}, for phospholipids, is the intensity of the reference line, which is taken as I_{722} or I_{1096}. I_{722} is a measure of the C-N symmetric stretch of the choline group, while the 1096 cm^{-1} line is attributed to the skeletal optical mode (C-C stretching), including PO_2^- symmetric stretching modes and *gauche* rotation. I_{CH_2} denotes the intensity ratio I_{2880}/I_{2850}, where I_{2850} is the intensity of the line due to the methylene symmetric C-H stretch and I_{2880} that due to the methylene asymmetric C-H stretch [2]. The constants in Eqn. 2 originate from I_{CH_2} values for crystalline and liquid hexadecane.

Both order parameters were interpreted as probabilities. S_T was introduced as a measure of the all-*trans* chain probability ($S_T = 1$ for crystalline dipalmitoyl phosphatidylcholine; $S_T = 0$ for no order). S_L was considered to be a measure of the closest packing probability ($S_L = 1$ for the closest possible packing, as in crystalline hexadecane; $S_L = 0$ for the completely disordered state, as in liquid hexadecane).

Obviously, in the choice of reference bands it was tacitly assumed that temperature does not affect the head-group vibrations and thus their contributions to I_{722} and I_{1096}. This implies on an entirely theoretical basis that hydrocarbon chain order-disorder transitions induced by interactions with polar head groups or those affecting the motion and the polarizability of the polar head groups cannot be included in Gaber and Peticolas' picture. Considerable deviations and inconclusive data can be expected in these cases, primarily in the S_T values. To demonstrate this, the effects of some simple ions in high concentrations on the structure of lipid-water dispersions were monitored by laser Raman spectroscopy.

The high-purity synthetic β,γ-dipalmitoyl-DL-α-phosphatidylcholine (Fluka A.G., Switzerland) used exhibited a single spot on a silica gel thin-layer chromatogram. Otherwise, analytical-grade purity chemicals (Reanal, Hungary) were used; these were dissolved in tridistilled water, without further purification. Sample preparation and measurement conditions are detailed elsewhere

[3]. Numerical values given below are averages of at least five independent measurements. The reproducibility of the spectra, even in the most noisy cases, was better than ±5%; all the bands used could be graphically evaluated with the same precision. The laser Raman spectra, in the relevant spectral regions, of dipalmitoyl phosphatidylcholine in the presence of different ions are shown in Fig. 1.

Both 722 and 1096 cm^{-1} lines were taken as reference in accordance with Gaber and Peticolas' suggestions. Intensity ratios and order parameters S_T (S_T^1 for $I_{ref} = I_{722}$ and S_T^2 for $I_{ref} = I_{1096}$) and S_L calculated via Eqns. 1 and 2 are listed in Table I. These data reveal that (a) the intensity ratios I_{1096}/I_{722} usually deviate from unity and, (b) the *trans* order parameters may exceed the theoretically predicted maximum value, the deviations being far above the inherent experimental measurement error. These imply that the structural changes induced by several ions in multi-bilayer systems cannot be conclusively described by the *trans* order parameters defined by Eqn. 1.

On the basis of the assignments of the Raman bands used in the calculations of S_T^1 and S_T^2, it is plausible that the anomalously high values of S_T for various ions are to be ascribed to the interactions between ions and the

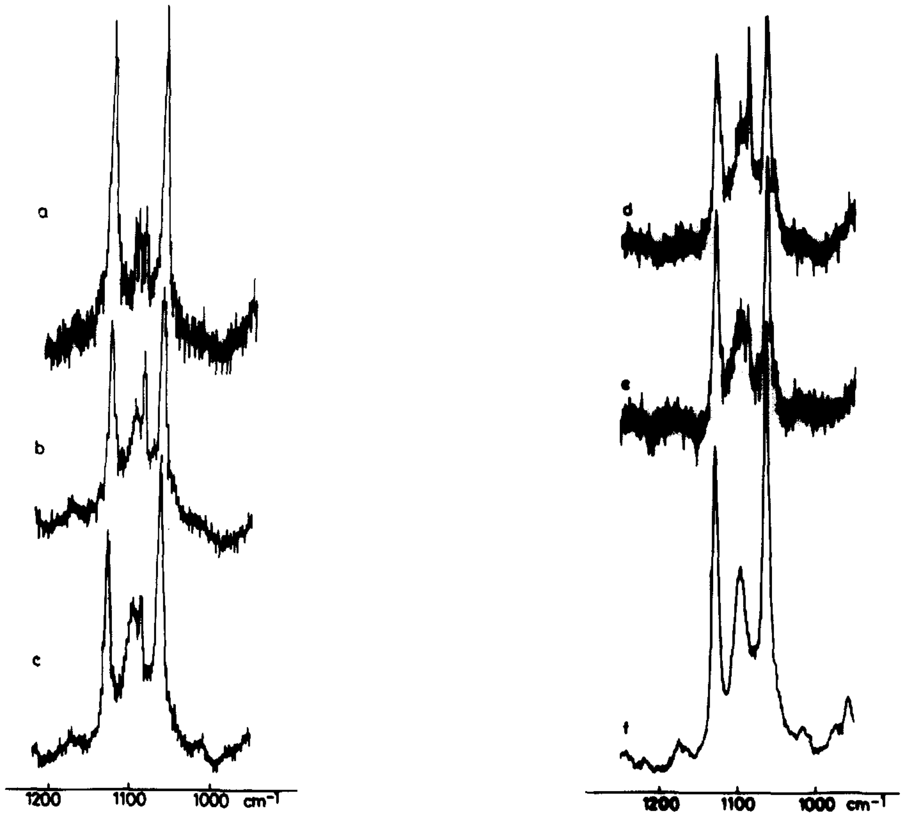

Fig. 1. Laser Raman spectra of dipalmitoyl phosphatidylcholine in lamellar aqueous dispersions containing different ions, in the Raman spectral range 1000—1200 cm^{-1}. a, 1 M $CaCl_2$; b, 1 M $MgCl_2$; c, 1 M $BaCl_2$; d, 1 M NaCl; e, 1 M $CdCl_2$; f, 10^{-2} M $Na_2S_2O_3$. Phosphatidylcholine:water = 1:4 (w/w).

TABLE I

CHARACTERISTIC INTENSITY RATIOS AND ORDER PARAMETERS (S_T^1 AND S_T^2 FOR THE ALL-*TRANS* CONFIGURATION OF HYDROCARBON CHAINS; S_L FOR THE LATERAL PACKING DENSITY) FOR DRY DIPALMITOYL PHOSPHATIDYLCHOLINE AND FOR AQUEOUS DISPERSION OF DIPALMITOYL PHOSPHATIDYLCHOLINE IN THE PRESENCE OF DIFFERENT ADDITIVES. PHOSPHATIDYLCHOLINE:WATER, 1:4 (w/w).

Composition of the dipalmitoyl phosphatidylcholine sample	$\frac{I_{1096}}{I_{722}}$	$\frac{I_{1130}}{I_{1096}}$	$\frac{I_{2880}}{I_{2850}}$	S_T^1	S_T^2	S_L
H_2O	1.20	1.45	1.28	0.99	0.97	0.39
1 M NaCl in H_2O	1.10	1.35	1.28	0.85	0.91	0.39
1 M KCl in H_2O	1.38	1.39	1.28	1.09	0.93	0.39
1 M RbCl in H_2O	1.45	1.45	1.28	1.19	0.97	0.39
1 M CsCl in H_2O	1.45	1.35	1.28	1.12	0.91	0.39
1 M $CaCl_2$ in H_2O	0.98	2.50	1.32	1.38	1.67	0.41
1 M $MgCl_2$ in H_2O	1.40	1.61	1.30	1.28	1.08	0.40
1 M $BaCl_2$ in H_2O	1.35	1.47	1.26	1.13	0.99	0.37
1 M $CdCl_2$ in H_2O	0.98	1.82	1.41	1.01	1.22	0.47
$1.5 \cdot 10^{-3}$ M I_2 in H_2O	1.28	1.18	1.16	0.86	0.79	0.31
10^{-2} M $Na_2S_2O_3$ in H_2O	1.49	1.61	1.32	1.36	1.08	0.41
Crystalline	1.18*	1.49	1.37	1.00	1.00	0.45

*In Gaber and Peticolas' paper [1] I_{1096}/I_{722} was considered as, to a good approximation, unity. Unfortunately, neither the spectrum reported in ref. 1, nor our most careful measurements made on 10 independent samples confirm the above approximate value. According to the latter the intensity ratio I_{1096}/I_{722} is 1.18 ± 0.02. Decomposition and evaluation of the Raman spectra by computer could provide somewhat more exact, but not significantly different intensity ratios.

zwitter-ionic polar head of phosphatidylcholine. The nature and points of attack of these interactions are not yet completely understood, but there is evidence [4] that they can change the hydration state of the phospholipid molecules. Such interactions certainly may affect the polarizability of the phosphoryl moiety, and are reflected in the Raman spectra of lipids as changes in the intensities of the corresponding bands. Such changes were, in fact, observed (Fig. 1).

As regards the lateral order parameter, S_L, no anomaly appeared. According to the S_L data, none of the salts considered loosened the packing density of the hydrocarbon tails, whereas molecular iodine did (Table I). This latter observation coincides with Szundi's recent results obtained in monolayer investigations (unpublished results), indicating the suitability and fairly high sensitivity of S_L for quantitative description of lateral order and packing density in aqueous lipid dispersions. Since the divalent ions considered have only a very slight ordering effect on the hydrocarbon moiety, it indicates that the ordering is probably due to secondary (perhaps head-group mediated) interactions ('tightening') or ion-head group interactions may equally be the cause of any apparent changes observed.

To summarize, it is shown on theoretical and experimental grounds that the *trans* order parameter defined in Eqn. 1 can only be applied to quantitative characterization of the all-*trans-gauche* relations in lipid systems if the polar head groups are not influenced by the external effect inducing the phase transition. If the perturbation of the polar heads of lipids results in a decrease of the polarizability (dehydration or partial dehydration [5]), S_T may exceed the theoretical maximum, unity, indicating that the polar head is also involved. Since S_T was originally considered as a probability, there is no reasonable way to interpret values higher than 1. Biological membranes consist of mixed phospholipids with varying saturation and chain length of the fatty acid residues. Therefore, further problems may arise if dipalmitoyl phosphatidylcholine, a saturated phospholipid with well-defined chain length, is chosen as reference. At the present stage of knowledge we are not in the position to propose a correct method of estimation of *trans* order in either membrane models or biological membranes. Much more data and systematic investigations on lipid-water, lipid-lipid, lipid-ion, etc. interactions are needed in order to give even only semi-quantitative measures of *trans* order in biomembranes.

The authors thank Mr. B. Szalontai for valuable and helpful comments on the manuscript. This work was supported by the Hungarian Academy of Sciences and by a UNESCO/UNDP Grant No. HUN/71/506/B/01/13 to Hungary.

References

1 Gaber, B.P. and Peticolas, W.L. (1977) Biochim. Biophys. Acta 465, 260—274
2 Spiker, Jr., R.C. and Levin, I.M. (1975) Biochim. Biophys. Acta 388, 361—373
3 Loshchilova, E. and Karvaly, B. (1977) Chem. Phys. Lipids 19, 159—168
4 Godin, N.G. and Ng, T.W. (1974) Mol. Pharmacol. 9, 802—819
5 Karvaly, B., Szundi, I. and Nagy, K. (1975) Bioelectrochem. Bioenerg. 2, 339—347