Chiral recognition of odorants (+)- and (-)-carvone by phospholipid monolayers

ARTICLE *in* JOURNAL OF THE AMERICAN CHEMICAL SOCIETY · FEBRUARY 1992

Impact Factor: 12.11 · DOI: 10.1021/ja00030a041

4 AUTHORS, INCLUDING:

William Neely
Auburn University
55 PUBLICATIONS **599** CITATIONS
SEE PROFILE

Vitaly Vodyanoy
Auburn University
227 PUBLICATIONS **2,010** CITATIONS
SEE PROFILE

Chiral Recognition of Odorants (+)- and (-)-Carvone by Phospholipid Monolayers

Suram Pathirana,[†] William C. Neely,[†] Lawrence J. Myers,[‡] and Vitaly Vodyanoy*[,‡]

Contribution from the Department of Chemistry, the Institute for Biological Detection Systems, and College of Veterinary Medicine, Auburn University, Auburn, Alabama 36849.
Received August 7, 1991

Abstract: Interactions of the odorants (+)- and (-)-carvone with L-α-1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (L-DPPC) monolayers were studied using surface pressure and surface potential versus area isotherms measured in the temperature range 10-35 °C in the presence and absence of 5 mM odorants in the subphase. The data indicate that the L-DPPC monolayers with (-)-carvone absorb twice as much heat as those with (+)-carvone when compressed at 30 °C. Under the same conditions, monolayers with (-)-carvone undergo a larger entropy change than monolayers with (+)-carvone. The temperature dependence of surface potentials of compressed L-DPPC monolayers exposed to (-)-carvone differs significantly from those exposed to the + isomer. Variations in thermodynamic properties and orientations of molecules within lipid assemblies may contribute to membrane recognition of optical isomers.

Molecular recognition is a very important concept in many biological processes. The olfactory systems of humans and animals can discriminate between odorants with a high degree of molecular similarity, e.g., between optical isomers.[1] It has been shown that lipid membrane components of several cell types are affected by the presence of odorant molecules.[2] In a previous report[3] it was demonstrated that small odorant molecules, as exemplified by cyclohexanone, were able to modify the thermodynamic properties of phospholipid monolayers. In this work, we have examined the effects of two enantiomeric molecules of an odorant, carvone, on the steady-state properties of L-DPPC monolayers as an in vitro model for chiral recognition in membranes. Arnett and coworkers[4] have clearly demonstrated that the chiral recognition in monolayers depends strongly on the ability of interacted molecules to bring their chiral centers into a favorable stereospecific interaction. Orientations of relatively large amphiphilic molecules in monolayers are restricted by strong interactions with a subphase so that favorable orientations resulting in stereoselectivity cannot always be accomplished. L-DPPC monolayers do not usually exhibit the molecular chirality because to their chiral centers are hidden inside hydrocarbon tails,[4,5] and only a strong structural perturbation can produce significant stereoselective interactions.[6] To increase the orientational freedom of chiral molecules in monolayers, these experiments use relatively small semipolar molecules of carvone which are able to penetrate phospholipid monolayer[3,7] and interact with chiral centers of L-DPPC.

Methods

Synthetic crystalline 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (L-α-dipalmitoyllecithin) (L-DPPC) (purity >99%) was obtained from Avanti Polar Lipids, Inc., (*S*)-(+) and (*R*)-(-)-carvone (5-ispropenyl-2-methyl-2-cyclohexenone) were obtained from Fluka (purity >99%). The odorants were analyzed for purity by mass spectrometry. The purity of the L-DPPC was examined by HPLC and IR spectroscopy. The subphase used in the standard experiments was a solution containing 55 mM KCl, 4 mM NaCl, 0.1 mM $CaCl_2$, 1 mM $MgCl_2$, and 2 mM 3-(*N*-morpholino)propanesulfonic acid (MOPS) made with deionized doubly distilled water (pH adjusted to 7.4 with 1 N KOH). For the experiments with the odorants a premixed solution of the odorant in 2% ethanol was dissolved in the above subphase. The lipid solutions were prepared in an atmosphere of dry nitrogen. L-DPPC was spread in a Teflon trough (45 cm × 15 cm) using a hexane (anhydrous, Aldrich) solution (50 μL of 1 mg ml^{-1}) containing 2% ethanol. The exact concentrations of spreading solutions were determined by a phosphorus assay. Each monolayer was allowed to equilibrate and to stabilize to 10 min before data collection. Surface pressure–surface area (Π-*A*) isotherms were measured at 10, 15, 20, 25, 30, and 35 °C. Using the minimum dispersion of mean molecular area as a criterion of the optimal rate of compression,[3] 0.5 cm^2 s^{-1} was chosen for these experiments. Control experiments with spreading solvents after evaporation gave no measurable change in surface pressure.[4] Each isotherm was replicated three to five times. Thermodynamic values of free energy (ΔG), entropy (ΔS), enthalpy (ΔH), and elasticity (E) for isothermal compression were calculated for pure L-DPPC monolayers and monolayers spread on subphases containing 5 mM (+)- or (-)-carvone (L-DPPC-(+)-carvone and L-DPPC-(-)-carvone monolayers). Apparatus for measurements of surface pressure and potential isotherms, methods of monolayer deposition, and data analysis were as described elsewhere.[3,8]

Results

The surface pressure–area isotherms obtained at five different temperatures between 10 and 35 °C for L-DPPC monolayers made on an odorant-free subphase replicate published data.[3,4,9] Parts A and B of Figure 1 show isotherms of L-DPPC monolayers spread on subphases containing 5 mM (+)- and (-)-carvone, respectively.

Table I shows the enthalpy and entropy changes for the compression between 0 and 30 mN/m, as a function of temperature, for the L-DPPC monolayers with (+)- and (-)-carvone, respectively. Using a 95% confidence limit as the criterion, the most significant chiral recognition effect was seen at 27.5 °C. At this temperature, L-DPPC monolayers with (-)-carvone absorb twice as much heat as monolayers with (+)-carvone. Under the same conditions, L-DPPC monolayers with (-)-carvone undergo a larger negative entropy change than monolayers with (+)-carvone.

Temperature dependences of differences in free energy of compression (ΔG) and surface potential (ΔV) (Figure 2) are similar. They are not monotonic, but exhibit a maximum around 293 K and a minimum around 300 K.

Discussion

The presence of odorant causes a significant difference in the shape and position of the isotherms (Figure 1). This indicates

[†] Department of Chemistry.
[‡] Institute of Biological Detection Systems.

(1) Leitereg, T. J.; Guadagni, D. G.; Harris, J.; Mon, T. R.; Teranishi, R. *Nature* **1971**, *230*, 455-456.
(2) Kashiwayanagi, M.; Kimie, S.; Kurihara, K. J. *Gen. Physiol.* **1987**, *89*, 443-457.
(3) Ito, H.; Morton, T. H.; Vodyanoy, V. *Thin Solid Films* **1989**, *180*, 1-13.
(4) Arnett, E. M.; Gold, J. M. *J. Am. Chem. Soc.* **1982**, *104*, 636-639.
(5) Arnett, E. M.; Gold, J.; Harvey, N.; Johnson, E. A.; Whitesell, L. G. In *Biotechnological applications of lipid microstructures*; Gaber, B. P., Schnur, J. M., Chapman, D., Eds.; Plenum Press: New York, 1988; pp 21-36.
(6) Weiss, R. M.; McConnell, H. M. *Nature* **1984**, *310*, 47-49.
(7) De Boeck, H.; Zidovetzki, R. *Biochim. Biophys. Acta* **1988**, *946*, 244-252.
(8) Vodyanoy, V.; Bluestone, G. L.; Longmuir, K. J. *Biochim. Biophys. Acta* **1990**, *1047*, 284-289.
(9) Phillips, M. C.; Chapman, D. *Biochim. Biophys. Acta* **1968**, *163*, 301-313.

0002-7863/92/1514-1404$03.00/0

Table I. Thermodynamic Properties for the Compression Process of the Monolayers at the Air–Water Interface for Pure L-DPPC Monolayers and for L-DPPC Monolayers with (+)- and (−)-Carvones Calculated[3,8] from Surface Pressure–Area Isotherms[a]

	$-\Delta H$ (kcal mol^{-1})						$-\Delta S$ (cal mol^{-1} K^{-1})					
ΔT (°C)	XC[b]	SD	C (+)[c]	SD	C (−)[d]	SD	XC[b]	SD	C (+)[c]	SD	C (−)[d]	SD
10–15	3.65	0.74	−2.42	3.34	9.17	2.35	21.58	2.62	7.00	11.70	41.2	8.23
15–20	4.96	2.44	10.75	3.35	15.56	2.22	26.13	8.44	45.80	14.60	63.4	7.69
20–25	17.99	2.96	15.14	4.93	14.27	5.04	70.61	10.04	60.80	16.83	59.0	17.02
25–30	15.53	2.56	14.24	4.03	29.35	5.01	62.32	8.52	57.80	13.41	109.6	16.94
30–35	8.41	5.19	14.61	2.14	16.14	2.14	38.82	17.46	59.00	7.02	66.0	7.09

[a] Change in entropy (ΔS) at a pressure of 30 mN/m and change in enthalpy (ΔH) for the compression between 0 and 30 mN/m as functions of temperature. Results are expressed as means ± SD of values measured in three or four experiments. Entropies (ΔS) were calculated at mean temperatures of intervals (ΔT). [b] XC = buffered subphase containing no carvones. [c] C (+) = 5 mM (+)-carvone in the subphase. [d] C (−) = 5 mM (−)-carvone in the subphase.

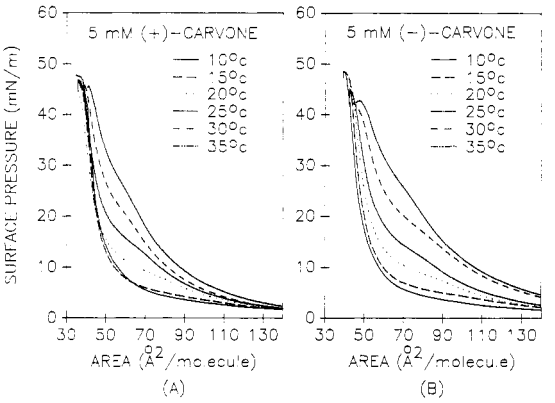

Figure 1. Surface pressure–area isotherms of L-DPPC on buffered subphase solutions (A) with 5 mM (+)-carvone and (B) with 5 mM (−)-carvone at the indicated temperatures. Each isotherm was obtained by averaging three or four runs. Standard deviations of the surface pressure were determined at 11 equally spaced points along the curves at each temperature. Mean standard deviations at 10, 15, 20, 25, 30, and 35 °C are as follows: (A) 0.311, 0.188, 0.174, 0.338, 0.342, 0.307 mN/m; (B) 0.249, 0.117, 0.064, 0.357, 0.097, 0.199 mN/m.

that these odorants interact with L-DPPC monolayers appreciably. Isotherms with the enantiomeric odorants show a clear chiral discrimination effect which is temperature dependent. Above 25 °C, L-DPPC monolayers with (−)-carvone are more expanded than monolayers with (+)-carvone. This may be attributed to different packing arrangements of L-DPPC in monolayers exposed to different odorants.

The thermodynamic properties (Table I) indicate that (−)-carvone interacts more strongly with L-DPPC than (+)-carvone. We suggest that (−)-carvone molecules have a stereospecific advantage over (+)-carvone molecules when they interact with L-DPPC monolayers. Thus, the (−)-carvone imposes greater restrictions on the motions of hydrocarbon chains, decreasing configuration freedom, with a consequent decrease in entropy.[3,7]

The data of Figure 2 indicate that (a) the average orientation of carvone molecules is temperature dependent and stereoselective; (b) the contribution of electrostatic dipole energy is large enough to modulate the free energy of the system; and (c) (−)- and (+)-carvone molecules make different contributions of dipole electrostatic energy to the free energy of the system.[3]

Conclusions

The results obtained indicate a significant chiral discrimination between enantiomeric odorants (+)- and (−)-carvone by monolayers of L-DPPC. The (−)-carvone interacts more strongly with L-DPPC than its enantiomer. These results are in agreement with

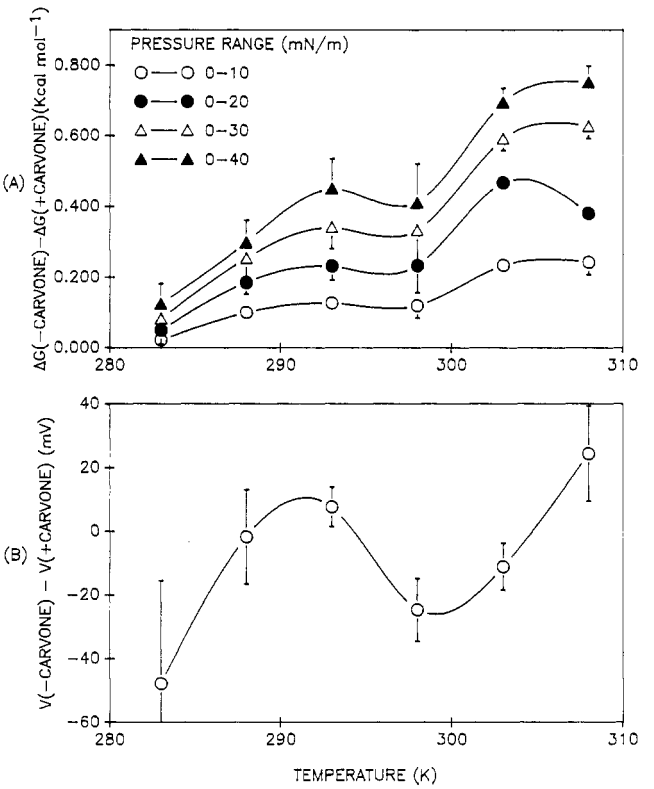

Figure 2. Differences in free energy of compression (ΔG) and surface potential (ΔV) between L-DPPC monolayers made on subpase containing 5 mM (−)- and (+)-carvone as functions of temperature. Differences in free energy (A) calculated[3] for indicated pressure ranges; differences in surface potential (B) measured[3] for fully compressed monolayers. Points represent mean values of experimental data (±SD) while curves are cubic spline interpolations.

the finding that (−)-carvones are much stronger odorants than (+)-carvones on a threshold basis.[1] The possibility of an important role of the phospholipid component of olfactory receptor membrane in olfaction does not necessarily conflict with the idea of chemospecific membrane proteins[10] but does offer an added dimension to the complex question of olfactory detection.

Acknowledgment. Supported by the U.S. Army (Grant DAAL03-90-G-009, V.V.) and Office of Naval Research (Grant N00014-90-J-1515, W.C.N., S.P.).

(10) Buck, L.; Axel, R. *Cell* **1991**, *65*, 175–187.