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Specific Molecular Properties of Organic Solvents Determine the Fluorescence Depolarization of DPH and TMA–DPH in Membranes

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In pig liver microsomes and protein-free egg PC liposomes the effects of organic solvent molecules on the fluorescence depolarization of 1,6-diphenyl-1,3,5-hexatriene (DPH) and 1-[4-(trimethylamino)phenyl]-6-phenyl-hexa-3,5-triene (TMA–DPH) were investigated. Aromaticity, alkyl chain length, and stereometry of the solvent molecules are shown to determine the changes of fluorescence depolarization. A concentration-dependent decrease in the fluorescence anisotropy is obtained with aromatic molecules but not with aliphatic molecules. The decrease correlates with the increasing side chain length of alkylbenzenes for both fluorophors and both membrane systems. Benzene in microsomes deviates characteristically from this trend. Measurements of TMA–DPH reveal smaller changes of anisotropy but yield the same qualitative results. Moreover, it is possible to distinguish the effects of the different stereometric properties of the three xylene isomers on the fluorescence anisotropy of DPH. According to their alkyl chain length and their specific stereometry, they exert the strongest fluidizing effect of all solvents investigated.

KEY WORDS: DPH; TMA–DPH; organic solvents; membranes; fluorescence depolarization.

INTRODUCTION

Organic solvent molecules are known to fluidize biological and artificial membrane systems. Usually this is connected with activity changes of membrane integral enzymes. Mostly it is not known whether these changes are due to a direct interaction of a membrane protein with the xenobiotic molecule (site specific interaction) or if the protein activity is changed by the membrane fluidization or another physical effect on the bulk membrane which is caused by the xenobiotic (unspecific indirect interaction). In our attempts to discriminate between these hypotheses, we looked for a membrane fluidity sensing method with a high resolving power for the effects of molecules of similar configuration. As xenobiotics we chose *n*-hexane, cyclohexane, benzene, toluene (methylbenzene), the three dimethylbenzenes *p*-xylene, *m*-xylene, *o*-xylene, and ethylbenzene. These molecules differ in their aromatic, aliphatic, and stereometric properties. As fluidity sensors DPH and TMA-DPH were used and their fluorescence anisotropy was measured. The membrane systems used were pig liver microsomes and protein-free liposomes prepared from egg phosphatidylcholine (PC).

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MATERIALS AND METHODS

Pig liver microsomes were prepared by means of differential centrifugation following the method of Lu [2], suspended in 0.01 *M* potassium phosphate buffer, pH 7.7, and stored at −20°C. Liposomes were prepared from egg phospatidylcholine by a standard method of

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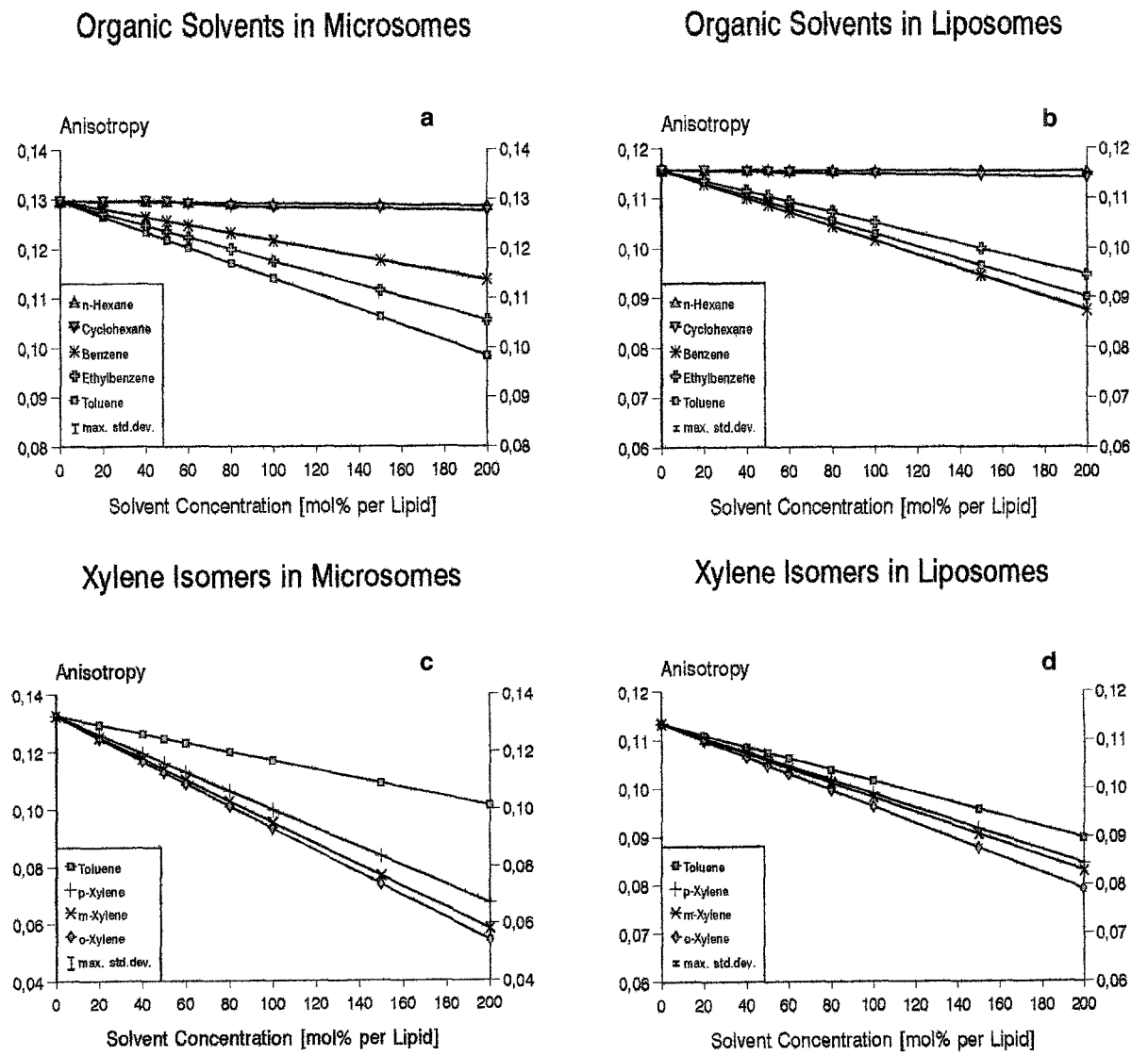

Fig. 1. DPH fluorescence anisotropy as a function of the molar membrane solvent to-lipid ratio. Anisotropy values are normalized to solvent-free assays. Mean standard deviations as indicated. (a,b) Microsomal assay; (c,d) liposomal assay.

Sartor *et al.* [4]. Fluorophors and solvents were diluted in stock solutions of dimethylformanide (0.5 m*M*) and ethanol (0.5 *M*), respectively. Fluorescence anisotropy did not depend on the ethanol concentration. The molar ratio of solvent to lipid in the membranes was calculated from partition coefficients and the volume ratio of solvent and buffer. The content of protein in microsomes and the content of phospholipid in liposomes were determined for each preparation according to the methods of Peterson [3] and Steward [6], respectively. The suspensions of microsomes and liposomes were incubated with 1,6-diphenyl-1,3,5-hexatriene (DPH), 1-[4-(trimethylamino)phenyl]-6-phenyl-hexa-1,3,5-triene (TMA–DPH) (Aldrich, 98%, Lambda) and with solvents for 90 min. The final concentration of DPH in the membranes was less than 0.4 mol% per lipid. The fluorescence polarization was measured with a Kontron-SFM25 spectrometer equipped with 2-nm slits. Excitation and emission wavelength were 358 and 428 nm, respectively, for both fluorophores. Fluorescence intensities of all probes were corrected for light scattering by subtracting the background (<5%) of control samples [5]. Measurements with microsomes were also corrected for light scattering by diluting the membrane suspensions [1]. Anisotropy measurements were performed at 25°C three times for each solvent.

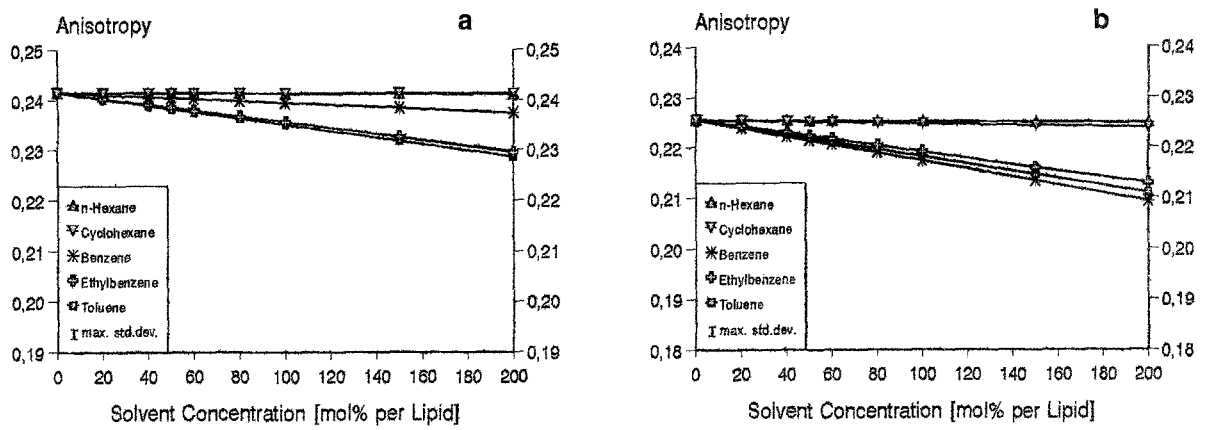

Fig. 2. TMA–DPH fluorescence anisotropy in microsomes (a) and liposomes (b). Representation as for Fig. 1.

RESULTS

Figures 1a and b show a decrease in the DPH fluorescence anisotropy with increasing concentrations of aromatic solvent molecules, but no change is obtained with the aliphatics *n*-hexane and cyclohexane. This holds for pig liver microsomes as well as for egg PC liposomes. The decrease in fluorescence anisotropy of the aromatic molecules is less pronounced with increasing length of the alkyl chain. In microsomes deviating from this trend, the effect of benzene is smaller than that of ethylbenzene and toluene. The measurements with TMA–DPH revealed smaller anisotropy changes than with DPH but the results are qualitatively identical (Figs. 2a and b).

Figures 1c and d show the DPH fluorescence anisotropy in the presence of increasing concentrations of the xylene isomers in microsomes and in egg PC liposomes, respectively. The effect is greater than that of all other molecules investigated and it increases from *p*- to *m*- to *o*-xylene.

DISCUSSION

Aromatic molecules cause a concentration-dependent decrease in the fluorescence anisotropy of DPH in microsomes and in protein-free liposomes by increasing the free volume in the membranes. *n*-Hexane, on the contrary, fits to the alkyl chains and causes almost no disturbance of the membrane order. A very different effect is obtained for benzene and its aliphatic pendant cyclohexane. Cyclohexane prefers the energetically favorable "chair conformation," which enables it to intercalate between the acyl chains to some extent. But this alone cannot explain the effect. The aromatics and aliphatics probably prefer different localizations. So the DPH fluorescence depolarization may be more sensitive for the effects of aromatics.

With increasing alkyl chains the aromatic solvents have stronger hydrophobic interactions with the neighboring alkyl chains, fit better between them, and cause the DPH fluorescence depolarization to decrease from benzene to ethylbenzene. Contrarily, in microsomes benzene shows a smaller fluidizing effect than ethylbenzene. We suppose that benzene accumulates at the protein acyl chain interface, thus decreasing its concentration in the bulk lipid and exerting a smaller fluidizing effect than in liposomes.

Measurements with TMA–DPH, which is localized in the rigid polar region of the phosphate groups, show higher values but smaller changes of anisotropy; they yield the same qualitative results. The higher anisotropy values result from the stronger localization of the TMA–DPH compared to DPH. And its smaller anisotropy changes indicate that the induced membrane fluidization by the solvent molecules takes place mostly in the hydrophobic core of the membranes.

It is possible to distinguish the effects of the different stereometric properties of the xylene isomers on the fluorescence anisotropy of DPH. The degree of depolarization increases according to their ability to disturb the order of the acyl chains, from *p*-xylene to *m*-xylene to *o*-xylene. The steric arrangement of the second methyl group causes a stronger fluidizing effect for *m*- and *o*-xylene than for toluene. For the extended *p*-xylene, which should intercalate in a first approximation between the acyl chains like toluene and which should possess even a greater hydrophobic interaction potential, we expected a smaller fluidizing effect than for toluene. But

possibly the two methyl groups interact with two different acyl chains so that their immobilization is more short-lived and their mobility is greater than that of toluene and ethylbenzene. So the fluidizing effect of *p*-xylene may be more like that of benzene in liposomes. We conclude that stationary DPH fluorescence measurements are an adequate method to discriminate interaction types of zenobiotics in membranes.

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