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Interaction of an odorant lactone with model phospholipid bilayers and its strong fluidizing action in yeast membrane

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Abstract

Some odorant lactones are naturally present in fruits or in fermented products; they can also be used as food additives and can be produced by microorganisms at the industrial scale by biotechnological processes. γ-Decalactone was previously shown to have antimicrobial properties. We determined by infrared spectroscopy measurements that this compound rapidly diffused into model phospholipid bilayers (within 2 min), modifying the general physical state of a dimyristoyl-L-α-phosphatidylcholine (DMPC) film. In vivo, the lactone strongly increased membrane fluidity in the model yeast *Yarrowia lipolytica*, as evaluated by fluorescence anisotropy measurements. This effect was more important than that of benzyl alcohol, which is known as a fluidizing agent in living cells, and may explain the toxic action of γ-decalactone in microorganisms.

Keywords: Lactone; DMPC bilayers; Membrane fluidity; *Yarrowia lipolytica*

1. Introduction

Some lactonic compounds have odorant properties and they are widely distributed in nature. They are naturally present in fruits, in fermented products (Maga, 1976) and they are also used as food flavoring additives. γ-Decalactone, for example, has a peach-like odor and it is used industrially, as a "natural" aroma compound when it is produced through biotechnological processes by yeast cells (Aguedo et al., 2000). This compound has also recently been described for anticonvulsant properties (Coelho de Souza et al., 1997).

Generally, microorganisms and animal cells are able to metabolize lactones through the preliminary action of esterases or lipases (Khalameyzer et al., 1999; Adams et al., 1998; Enzelberger et al., 1997) but beyond a concentration threshold, they become toxic (Endrizzi-Joran, 1994). The antimicrobial properties of some lactones have been, in fact, reported (Scarselletti and Faull, 1994; Endrizzi-Joran, 1994). To our knowledge, the mechanisms underlying this toxicity have not yet been specified.

The dimorphic food spoilage yeast *Yarrowia lipolytica* is able to produce large amounts of γ-decalactone when growing in the presence of ricinoleic acid (Aguedo et al., 2000), making this species a good model to study lactone toxicity mechanisms. According to the hydrophobic nature of γ-decalactone, it may interact with cell membranes: in order to evaluate this

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point, in the present study, the interaction kinetic of γ-decalactone with model phospholipid bilayers was evaluated using infrared spectroscopy. The consequent action on biological membranes was determined in vivo by fluorescence anisotropy measurements and the effect of the lactone were compared to that of the fluidizing agent benzyl alcohol (Coster and Laver, 1986).

2. Material and methods

2.1. Strain and culture conditions

Y. lipolytica W29 (ATCC20460; CLIB89) was grown in a glucose medium in the conditions previously described (Waché et al., 2000). Late exponential phase cells (theoretical OD_{600} of 4) were harvested by centrifugation (6000 × *g*, 5 min, 4 °C), washed twice in physiologic water (9 g l^{-1} NaCl in distilled water) and were then kept at 4 °C until used for fluorescence measurements.

2.2. Fluorescence polarization measurements with DPH

Membrane fluidity was assessed by measuring fluorescence anisotropy of 1,6-diphenyl-1,3,5-hexatriene (DPH) (Sigma, St. Quentin Fallavier, France). The experiments were conducted with whole yeast cells as reported by Laroche et al. (2001). The measurements were done with a spectrofluorometer (Hitachi Instrument, F4500, Japan) equipped with a stirred and thermostated (27 °C) cuvette holder, and connected to an acquisition and processing system (Hitachi).

The excitation wavelength was set at 360 nm (slit width of 10 nm) and the emission at 450 nm (slit width of 20 nm). The measured fluorescence intensities were corrected for background fluorescence and light scattering from the unlabelled samples. Fluorescence anisotropy (*r*) was calculated as follows:

$$r = \frac{I_{VV} - GI_{VH}}{I_{VV} + 2GI_{VH}} \quad \text{and} \quad G = \frac{I_{HV}}{I_{HH}}$$

I_{VV} and I_{VH} are the fluorescence intensities determined at vertical and horizontal orientations of the emission polarizer when the excitation polarizer is set in the vertical position. This applies similarly for I_{HV} and I_{HH} with the horizontal excitation polarizer. *G* is a correction factor for background fluorescence and light scattering. The yeast cells suspension was diluted into physiologic water to obtain an OD_{600} of 1.4 in a 2-ml quartz cuvette. γ-Decalactone (Sigma), or benzyl alcohol (Fluka, St. Quentin Fallavier, France) from a 235-mM stock solution in ethanol, or ethanol alone for the reference, were added to the cell suspension and after 5 min, 4.5 μl of a 1.8-mM DPH solution in tetrahydrofuran were added in the cuvette. After a 20-min probe insertion period (this was found to be necessary to obtain stabilized *r* values), a minimum of four values for *r* were determined and a mean value was calculated.

2.3. ATR-FTIR studies

Attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FTIR) experiments were conducted on a Vector 22 FTIR spectrometer, data processing was done with the Opus software (both from Bruker, Karlsruhe, Germany) with a wavenumber accuracy of 0.1 cm^{-1}. The model phospholipid, i.e. dimyristoyl-L-α-phosphatidylcholine (DMPC) (Sigma), was deposited (150 μl) to form a solid film on IR-transparent ZnSe window from a 20 g l^{-1} chloroformic solution. Hydrated DMPC is known to undergo a gel to liquid phase transition at 23 °C (Bouchard et al., 1996). The film was slightly hydrated with 100 μl distilled water and then the window was covered with a water-tight cell, enabling the introduction of γ-decalactone solution on the lipid film. Temperature was measured precisely with a thermocouple inserted in the cell. γ-Decalactone solution (2 ml) was introduced into the cell within less than 10 s at 27 °C. At convenient times, five scans were taken (4 cm^{-1} resolution) and an average spectrum was produced. The maximum of the CH_2 symmetric and asymmetric stretching bands were then reported against time.

3. Results and discussion

The interaction of γ-decalactone with model phospholipid system was investigated by the mean of ATR-FTIR spectroscopy. C—H bond of methylene

groups in an acyl chain gives a typical symmetric stretching (ν_s CH_2) band at 2851 (+3) cm^{-1} and one asymmetric stretching (ν_a CH_2) band at 2920 (+4) cm^{-1} (Akyüz and Davies, 1998). From the ATR ZnSe crystal dimensions and from the number of DMPC molecules, the lipid film can be assimilated to a multibilayer composed of approximately two thousands superposed bilayers (Goormaghtigh et al., 1999). We reported ν_s CH_2 and ν_a CH_2 values against time, at 27 °C, from the moment when γ-decalactone (Fig. 1) solution was brought into contact with DMPC, at a molar ratio slightly inferior to one lactone for two lipids (Fig. 2). Therefore, the curves represent the diffusion of the lactone within this structure. As lactone was added to the model phospholipid, both wavenumbers shifted to higher values, indicating an increased global disorder degree within bilayers. First, there was a rapid insertion phase (within about 2 min) and then a slow and longer phase during which ν_a CH_2 (Fig. 2A) and ν_s CH_2 (Fig. 2B) values slightly reached a maximal value: this may correspond to an equilibrium phase. When pure water was introduced onto DMPC bilayers, there was no shift in ν_a CH_2 and ν_s CH_2 wavenumbers; these remained equal to the values obtained before the introduction of the γ-decalactone solution (data not shown). Thus, the detected shifts correspond only to the introduction of lactone within DMPC.

The CH_2 stretching signals give information about the physical state of a lipid bilayer (Lewis and McElhaney, 1998). Here, we used FTIR measurements to evaluate the kinetics of interaction of γ-decalactone with DMPC bilayers, and thereby to evaluate the kinetics of variation in DMPC mobility following the introduction of a foreign compound in the bilayers. This first experiment clearly showed that γ-decalactone enhanced the fluidity of DMPC bilayers. For such an effect, γ-decalactone can be related to some other odorant compounds, even those that are chemically different from lactones and are reported to affect the physical state of model membranes, i.e. β-ionone and menthone (Bouchard et al., 1996) or citral and amyl acetate (Enomoto et al., 1991). The interactions between these compounds and membranes were proposed to account for the increase in membrane fluidity observed in vivo—for example, with the insecticides DDT (Donato et al., 1997) and lindane (Antunes-Madeira and Madeira, 1989), leading to cell toxicity, as observed for β-pinene (Uribe et al., 1985).

Fig. 1. γ-Decalactone structure.

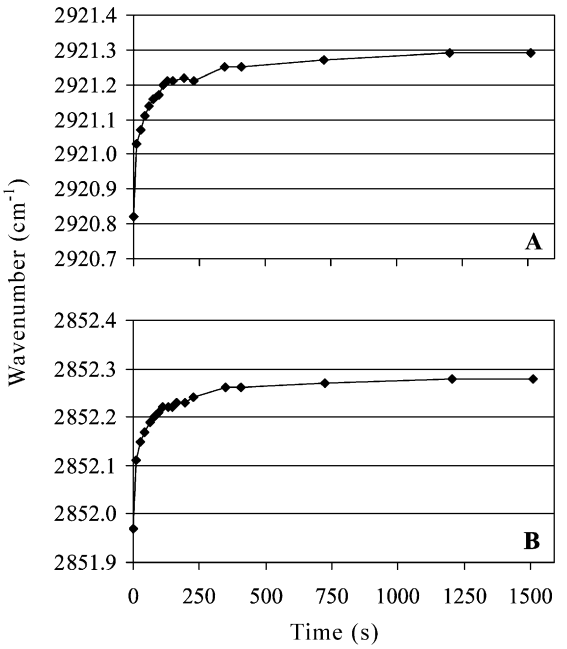

Fig. 2. Monitoring at 27 °C of CH_2 asymmetric (A) and symmetric (B) stretching vibration frequencies of DMPC bilayers brought into contact with γ-decalactone (1 mM) at a time equal to 0. These curves are representative of the interaction, the standard deviation for each point on y values is inferior to ± 0.05.

In the second part of this study, the interaction of γ-decalactone with a biological membrane was studied by fluorescence spectroscopy. We evaluated the in vivo action of γ-decalactone by fluorescence anisotropy measurements of DPH introduced into membranes of the model yeast *Y. lipolytica*. Fluorescence anisotropy (r) gives an inverse indication of membrane fluidity, i.e. a decreasing value of r indicates an increased disorder or "fluidity" (Shechter, 1997). Results proposed in Fig. 3 showed that γ-decalactone strongly modified fluorescence anisotropy for all tested concentrations. γ-Decalactone

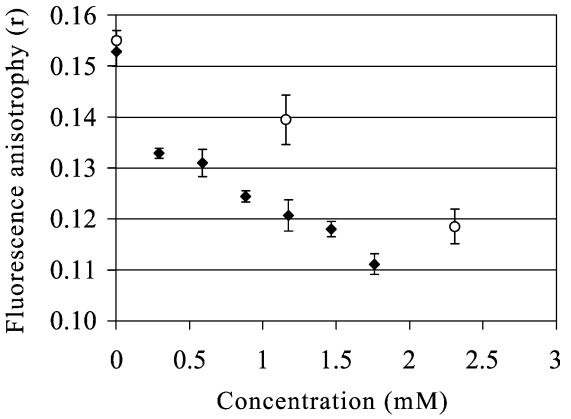

Fig. 3. Fluorescence anisotropy of yeast cells evaluated at 27 °C in the presence of γ-decalactone (♦) or benzyl alcohol (○). The final concentrations of γ-decalactone and benzyl alcohol in medium are indicated on the graph. For each compound, the reference and all the assays contained the same ethanol concentration (corresponding to the ethanol content of the highest tested concentration of the compounds), i.e. 105 mM for γ-decalactone and 151 mM for benzyl alcohol (representing 0.48% and 0.69%, respectively, of the total volume). Data were calculated using at least four r values determined in three measurement series from two distinct experiments.

influence was in a concentration-dependent manner between 0.3 and 1.8 mM. Comparison between the effects of γ-decalactone and the well-known fluidizing agent benzyl alcohol (Konopásek et al., 2000; Coster and Laver, 1986) is also presented in Fig. 3. The r values of the concentrations of benzyl alcohol that were tested decreased almost linearly, reaching a value 0.12 for 2.3 mM. The results indicate that this last compound had a stronger fluidizing effect than benzyl alcohol for equal concentrations of both compounds. The ethanol present in the medium did not significantly influence r values and measurements done without ethanol gave $r = 0.155 \pm 0.001$, which agrees with the results from references presented here (Fig. 3).

In conclusion, FTIR experiments enabled the detection of the disordering effect of the incorporation of a small hydrophobic compound within model phospholipid multibilayers, and kinetics measurements showed that the interaction was rapid. These data were obtained at 27 °C, with liquid-crystalline lipids which corresponds to the physical state of membrane phospholipids in physiological conditions in yeast and more generally in living systems. These results may be explained by a strong interaction between the hydrophobic carbon chain of γ-decalactone (Fig. 1) with that of the acyl chains of phospholipids. This interaction may constitute a major element contributing to the observed antimicrobial action of γ-decalactone (Endrizzi-Joran, 1994), the increase in membrane fluidity may for example be associated with modifications in membrane permeability properties (Shechter, 1997) and in the activity of membrane proteins (Antunes-Madeira and Madeira, 1989).

These data bring about some elements, explaining the way the odorant γ-decalactone interacts with cell membranes. The fluidizing properties we describe can be useful to obtain controlled membrane physical state modifications in microorganisms, alternatively to benzyl alcohol that is currently used (Konopásek et al., 2000). Moreover, γ-decalactone and other odorant lactones have no known metabolic function in plant and yeast cells. The membrane fluidizing action we demonstrate in this study could constitute a natural function for these compounds.

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