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Dynamic structural changes in microbial membranes in response to high hydrostatic pressure analyzed using time-resolved fluorescence anisotropy measurement

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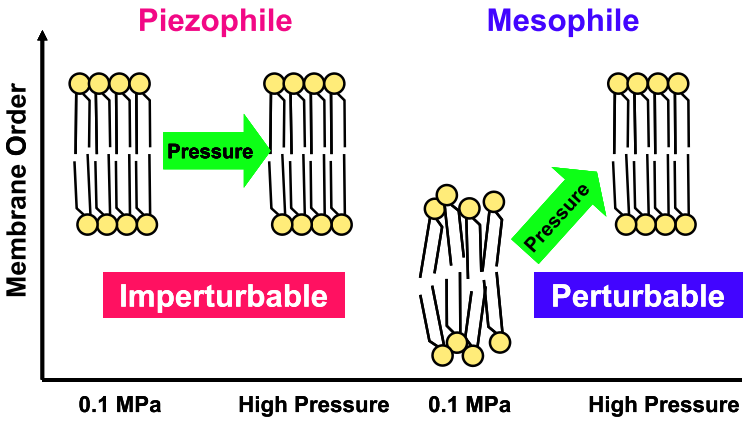
HIGHLIGHTS

- High hydrostatic pressure has a profound physiological impact on lipid membranes.
- Deep-sea organisms possess specialized cell membranes.
- This mini-review focuses on pressure-induced changes in microbial cell membranes.
- High-pressure time-resolved fluorescence anisotropy measurement is highlighted.

GRAPHICAL ABSTRACT



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ABSTRACT

High hydrostatic pressure has a profound physiological impact on lipid membranes, primarily resulting in tighter packing and restriction of acyl-chain motion. To fulfill membrane protein functions in high-pressure environments, deep-sea organisms possess specialized cell membranes. Although the effects of high-pressure on model membranes have been investigated in great detail, high-pressure-induced structural changes in living cell membranes remain to be elucidated. Of the spectroscopic techniques available to date, fluorescence anisotropy measurement is a common useful method that provides information on dynamic membrane properties. This mini-review focuses on pressure-induced changes in natural cell membranes, analyzed by means of high-pressure time-resolved fluorescence anisotropy measurement (HP-TRFAM). Specifically, the role of eicosapentaenoic acid in deep-sea piezophiles is described in terms of the structural integrity of the membrane under high pressure.

Abbreviations: HP-TRFAM, high-pressure time-resolved fluorescence anisotropy measurement; TCSPC, time-correlated single-photon counting; DPH, 1,6-diphenyl-1,3,5-hexatriene; TMA-DPH, 1-[4-(trimethylamino)pheny]-6-phenyl-1,3,5-hexatriene; *r*, fluorescence anisotropy; *S*, order parameter; D_w, rotational diffusion coefficient; SOPC, stearoyl-oleyl-phosphatidylcholine; DOPC, dioleyl-phosphatidylcholine; DPPC, dipalmitoyl-phosphatidylcholine; POPC, palmitoyl-oleoyl-phosphatidylcholine; DSPC, distearoyl-phosphatidylcholine; SOPC, stearoyl-oleyl-phosphatidylcholine; SAPC, stearoyl-arachidonoyl-phosphatidylcholine; SDPC, stearoyl-docosahexanoylphosphatidylcholine; PUFA, polyunsaturated fatty acids; EPA, eicosapentaenoic acid (C20:5); DHA, docosahexaenoic acid (C22:6).

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1. Introduction

Maintenance of structural and dynamic properties of cell membranes is required for fundamental physiological functions of organisms. High hydrostatic pressure and low temperature have a profound impact on lipid membranes, primarily resulting in tighter packing and restriction of acyl-chain motion. A number of excellent reviews of high-pressure effects on artificial lipid membranes has been published previously [1–5], and these should be consulted for a detailed biophysical basis of membranes, which will not be covered in this mini-review. Primarily, increasing hydrostatic pressure orders lipid membranes in a manner analogous to decreasing temperature. With increasing pressure, the gel-to-liquid crystalline coexistence region is shifted toward higher temperatures by approximately 22 °C/100 MPa [2]. For example, a pressure increase of 100 MPa increases the main transition (L_β/L_α) temperature of the stearoyl-oleyl-phosphatidylcholine (SOPC) and dioleylphosphatidylcholine (DOPC) membrane by 18.1 °C and 23.3 °C, respectively [6].

In organisms that live at atmospheric pressure, most physiological processes employed by membrane proteins such as respiration, nutrient import, ion flux, and signaling are compromised by high pressure and low temperature, at least in some cases, due to perturbation of the membranes [7–10]. It is commonly believed that deep-sea organisms have developed their membranes and membrane proteins to adapt to such extreme conditions. However, there are still a limited number of examples that have been proven experimentally. In a broad range of organisms, the packing effects of the membrane can be compensated by modifying the fatty acid compositions. Cold adaptation is often associated with the incorporation of unsaturated bonds within the acyl chains [11–13]. Membrane acyl chains containing unsaturated bond(s) assume a more bulky conformation than their saturated counterparts, allowing greater conformational freedom and less packing of the membrane. Consequently, the membrane becomes more fluid. This adaptation, employed by organisms inhabiting cold and high-pressure environments, has been termed "homeoviscous adaptation" [8,14,15]. Whether homeoviscous adaptation generally occurs in deep-sea organisms remains unknown because natural membrane properties in response to high pressure have not been sufficiently analyzed. In addition, the variability and complexity of natural cell membranes make it difficult to characterize how the physicochemical properties of the membranes respond to high-pressure conditions. Membrane fluidity and phase transitions can be analyzed by a variety of methods including differential scanning calorimetry, nuclear magnetic resonance, electron spin resonance, X-ray diffraction and fluorescence anisotropy measurement. Among these spectroscopic techniques, fluorescence anisotropy measurement using rod-like probes whose direction of absorption and emission transition moments coincide with the long molecular axis is a highly sensitive, simple method that provides information on lipid order and rotational motion of acyl chains [16–18]. This mini-review focuses on the effects of high hydrostatic pressure on the dynamic structure of the microbial membrane as analyzed by fluorescence anisotropy measurements.

2. Fluorescence anisotropy measurement of membranes under high pressure

Fluorescence anisotropy measurement has been widely employed for the study of model membranes. The lipophilic fluorescent probes, 1,6-diphenyl-1,3,5-hexatriene (DPH) and its cationic derivative 1-[4-(trimethylamino)pheny]-6-phenyl-1,3,5-hexatriene (TMA-DPH), are commonly used for such analyses. When added to model membranes or natural membranes, DPH primarily distributes perpendicular to the bilayer plane near the center of the membrane, but partially distributes parallel to it within the acyl chain tails [17]. The use of DPH in natural membranes requires special care because it can form granules in some cells even at low concentrations, making interpretation of the results difficult [19]. TMA-DPH is distributed to the lipid–water interface due to its charged moiety and thereby reflects only the interfacial region of the membrane. These probes should not disturb membrane dynamics and hence they are typically incorporated into model lipid bilayers in a molar ratio of 1/1000–1/500, or are used for labeling natural cell membranes at 0.5–5 μM.

Time-resolved fluorescence anisotropy measurement based on time-correlated single-photon counting (TCSPC) provides quantitative information on membrane order and rotational motion of acyl chains in a single measurement. Fluorescence anisotropy is described by Eq. (1), where I_{VV} and I_{VH} are the fluorescence intensities (the two subscripts indicate the orientation of the excitation and emission polarizer, respectively, with H indicating horizontal and *V* indicating vertical) and $G = I_{HV} / I_{HH}$ is the instrumental factor.

$$r(t) = [I_{VV}(t) - G\, I_{VH}(t)]/[I_{VV}(t) + 2G I_{VH}(t)]. \quad (1)$$

The simplest model of the restricted motion of fluorochromes in the membrane, based on the Brownian diffusion of the label in a cone with a wobbling diffusion constant, leads to the following single exponential approximation of the anisotropy decay with time, $r(t)$ [20]:

$$r(t) = (r_0 - r_\infty)\cdot \exp(-t/\theta) + r_\infty \quad (2)$$

where r_∞ stands for limiting anisotropy, and θ (ns) for rotational correlation time. The order parameter (S) is calculated to obtain structural information on the membrane according to the following equation:

$$S = (r_\infty / r_0)^{1/2}. \quad (3)$$

The rotational (wobbling) diffusion coefficient (D_w) is calculated to obtain the dynamic nature of the membrane according to the following equation:

$$D_w = (r_0 - r_\infty)/6\theta r_0. \quad (4)$$

3. Effects of high pressure on model membranes

In the pioneering study by Bernsdorff et al., time-resolved fluorescence anisotropy measurement on model membranes, dipalmitoylphosphatidylcholine (DPPC) and palmitoyl-oleoyl-phosphatidylcholine (POPC), was performed under high pressure using a high-pressure optical chamber [21]. At temperatures where the membranes remain in the liquid-crystalline phase, the order parameter *S* for the TMA-DPH-labeled DPPC and POPC membrane shows a sharp increase at around 75 MPa at 58 °C and 95 MPa at 15 °C, respectively. These increases in *S* correspond to the liquid-crystalline-to-gel phase transition. Addition of 30 mol% cholesterol to the membrane systems results in an increase in *S* and concomitant loss of the pressure-induced sharp phase transition in both DPPC and POPC membranes [21]. This behavior is attributable to the formation of homogeneous liquid ordered phase in the systems.

In our recent study, we also created a system that enabled *h*igh-*p*ressure *t*ime-*r*esolved *f*luorescence *a*nisotropy *m*easurement, namely HP-TRFAM, under high pressure, aimed at understanding the effects of high pressure on the cell membrane in deep-sea piezophiles (referred to hereafter) [22]. The HP-TRFAM system comprises of a high-pressure optical cell, a high-pressure pump, and a TCSPC device (Fig. 1A). The strain birefringence of the quartz windows is simply corrected by determining the r_0 values for DPH in mineral oil under various hydrostatic pressures. This is based on the fact that the r_0 values do not change by applying pressures at the experimental pressure range (<200 MPa). The measured r_0 of DPH is almost unchanged at pressures up to 50 MPa, but the birefringence of the window becomes considerable at pressures greater than 50 MPa (Fig. 1B). Thus, measured r_0 and r_∞ values are multiplied by the correction coefficients to determine the correct r_0 and r_∞ values at each pressure. Fig. 2 shows a typical example