Biochimica et Biophysica Acta 1818 (2012) 2850–2859

Contents lists available at SciVerse ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbamem

Effect of 2-(4-fluorophenylamino)-5-(2,4-dihydroxyphenyl)-1,3,4-thiadiazole on the molecular organisation and structural properties of the DPPC lipid multibilayers

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ARTICLE INFO

Article history:
Received 29 March 2012
Received in revised form 17 July 2012
Accepted 18 July 2012
Available online 24 July 2012

Keywords:
1,3,4-Thiadiazole
DPPC lipid
Multibilayer
X-ray diffraction
FTIR spectroscopy

ABSTRACT

Interactions and complex formation between lipids and biologically active compounds are crucial for better understanding of molecular mechanisms occurring in living cells. In this paper a molecular organisation and complex formation of 2-(4-fluorophenylamino)-5-(2,4-dihydroxybenzeno)-1,3,4-thiadiazole (FABT) in DPPC multibilayers are reported. The simplified pseudo binary phase diagram of this system was created based on the X-ray diffraction study and fourier transform infrared spectroscopic data. The detailed analysis of the refraction effect indicates a much higher concentration of FABT in the polar zones during phase transition. Both the lipid and the complex ripple after cooling. It was found that FABT occupied not only the hydrophilic zones of the lipid membranes but also partly occupied the central part of the non polar zone. The infrared spectroscopy study reveals that FABT strongly interact with hydrophilic (especially PO_2^-) and hydrophobic (especially "kink" vibrations of CH_2 group). The interactions of FABT molecules with these groups are responsible for changes of lipid multibilayers observed in X-ray diffraction study.

1. Introduction

The most important clinical problem related to the use of new therapeutic strategies is the high toxicity of new, potentially cytotoxic compounds. This is caused by the lack of clear biochemical differences between tumour and normal cells and non-specificity of the anti-tumour action of cytostatics [1,2].

High expectations for therapies are attached to the extensive ongoing research conducted on the group of 2-amino-1,3,4-thiadiazole substituted with 2,4-dihydroxyphenyl group [3–6]. The literature presents the variously-substituted 1,3,4-thiadiazoles as compounds with a broad spectrum of biological activity [7–9]. 2-(4-fluorophenylamino)-5-(2,4-dihydroxyphenyl)-1,3,4-thiadiazole, hereafter abbreviated (FABT), belongs to a large family of 2,4-dihydroxyphenylo-1,3,4-thiadiazoles which exhibit antiproliferative activity in vitro against human tumour cell lines: bladder cancer HCV29T, lung A549, colorectal SW707 and breast cancer T47D [3,10,11]. FABT has also fungistatic properties [5]. FABT molecules exhibit keto-enol tautomery with a significant equilibrium shift towards keto form in n-alkane solvents [12]. The structure of this compound was first time confirmed by structural crystallography [13] where FABT was crystallised in the ionic form.

It is believed that the FABT biological activity has to be partly related to its interactions with lipid membranes. It has been found that FABT does not only change the lipid layers dynamics and stiffness but can also form complexes with lipids similar to: benzothiazoles [14], α-tocopherol [15], dipalmitoylglycerol [16], polymers [17] and DNA [18]. Investigations of interactions between aminothiadiazoles with biological membranes as well as an analysis of the dynamics of these interactions may prove crucial for understanding these mechanisms.

The combination of X-ray diffraction and FTIR spectroscopy techniques facilitates the study of molecular organisation, multilayer dynamics, and lipid-FABT interactions in the lipid environment.

2. Materials and methods

The structure and crystallisation of 2-(4-fluorophenylamino)-5-(2,4-dihydroxybenzeno)-1,3,4-thiadiazole (**FABT**) are described by Kaminski at al. [13] and the synthesis of FABT and its derivatives can be found in the references [19,20]. The schematic structure is presented in Scheme 1. The purity of the compound was 99%. The purity of the lipid dipalmitoylphosphatidylcholine (DPPC) was 99%; therefore, it was used without further purification. All the solvents used were purchased from Sigma Chem. Co. (USA).

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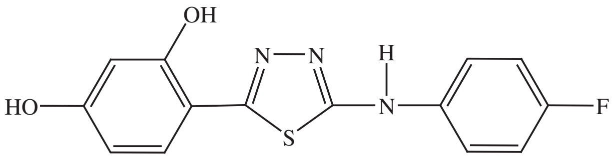
doi:10.1016/j.bbamem.2012.07.013



Scheme 1. FABT molecule.

3. Multilayer preparation

The sample preparation procedure is very similar to the one described in references [14,21]. First, the lipids were dissolved in chloroform at a concentration of 1×10^{-2} M. The FABT was dissolved in methanol at a concentration of 5×10^{-3} M. Both solutions were mixed in a 2 mL vial and the solvents were evaporated under a N_2 stream and then under vacuum for ~2 h. After complete solvent evaporation, 0.5 mL of ultrapure water was added and the mixture was homogenised with a sonicator (Bandelin Sonoplus) 3× for 1 s with 60% of total power. Finally, the mixture was deposited on mica plates. Afterwards, the samples were left for 24 h at 24 °C in N_2 atmosphere until complete water evaporation. The multibilayers thus prepared were subjected to the X-ray investigation. In the case of FTIR technique, the water mixture was deposited on the Ge attenuated total reflectance (ATR) crystal.

4. X-ray measurements

X-ray diffraction patterns were collected with a Bruker D8 Discover system, working in the reflection mode. A parallel beam of CuKα radiation (line focus) was formed by Gebel mirror, and the diffracted intensity was controlled with a scintillation counter. Sample temperature was stabilised by the Anton Parr DCS350 heating stage (with accuracy 0.1 °C). Precision sample positioning was assured by the Eulerian cradle mounted on the goniometer.

After alignment the samples were heated from 25 °C to 70 °C and then cooled to 25 °C with temperature step of 1 °C. The X-ray patterns were recorded after temperature stabilisation. The relative humidity of air in experimental hutch was around 30%.

5. FTIR measurements

Infrared absorption spectra were recorded with a 670-IR Varian spectrometer. The attenuated total reflection (ATR) configuration was used with 10 internal reflections of the ATR Ge crystal plate (45° cut). Typically, 25 scans were collected, Fourier-transformed and averaged for each measurement. Absorption spectra at a resolution of one data point per 1 cm^{-1} were obtained in the region between 4000 and 600 cm^{-1}. The instrument was continuously purged with N_2 for 40 min. before and during measurements. The Ge crystal plate was cleaned with ultra pure organic solvents from Sigma-Aldrich. The spectral analysis was performed with Grams/AI software from ThermoGalactic Industries (USA).

6. Modelling of multibilayers

The reflection intensity was corrected by standard factors (Lorenz, polarisation and active area) [22]. The peak positions were corrected for the refraction effect according to Eq. (1) presented in a previous paper [23] because a small deviation from Bragg law were observed for small angles.

$$N\lambda = 2d\sqrt{\sin^2\theta - 2\bar{\delta}}, \tag{1}$$

where:

$$\bar{\delta} = x\delta_1 + (1-x)\delta_2 + \frac{1}{2}x(1-x)\left(\sqrt{\sin^2\theta-2\delta_1} - \sqrt{\sin^2\theta-2\delta_2}\right)^2,$$

N order of reflection, λ wave length, θ angle of incident X-ray beam, d interlayer spacing, $x = d_1/d$ where d_1 is the thickness of a lipid layer with the refraction index n_1 (see bottom part of Fig. 6), $\delta_1 = 1 - n_1$ and $\delta_2 = 1 - n_2$. In this case, the following parameters were fixed: $d_1 = 20$ Å and $n_1 = 0.999999$. The following parameters related to refraction were fitted: the refraction index n_2 and interlayer spacing d. Because refraction depends in this case on the ratio between refraction indices n_1 and n_2, the n_1/n_2 ratio is presented. The refraction indices change at the level of 1×10^{-6}, which is expected in the case of soft matter for X-ray diffraction.

The diffractometer alignment can have an effect on refraction correction but in this case clear effects from sample are observed. The refraction correction was not very important for pure lipids; thus it was reduced to the simple form of Bragg equation. In the present paper, the momentum transfer vector $\vec{q}$ is related to the interlayer spacing $\vec{d}$ by the equation:

$$\vec{q} = \frac{2\pi}{\vec{d}}.$$

The lipid electron density model is built of four Gauss functions:

$$\rho_{layer}(z) = \sum_{i=1}^{4} A_i e^{-\left(\frac{z-d_i}{<u^2>}\right)^2} \frac{1}{\sqrt{2\pi<u^2>}},$$

with an amplitude of A_i and positions of d_i: 0, yd, $1/2d$ and $(1-y)d$ in a one-dimensional unit cell. In this case, y describes the position of Gauss maximum in fractional coordinates in the lipid 1D unit cell and can have values in the range from 0 to 0.5.

Here, the $<u^2>$ is the average square displacement parameter of the group of atoms described by Gauss functions and was fixed at 3.8 Å^2, which is slightly higher than the value suggested by the authors in the reference [24].The Gauss function amplitudes were fitted to obtain the best fit to the data using the χ^2 method. Despite its simplicity, such a model describes the data well and gives consistent results to the other one from literature [25,26]. The total electron density profile is obtained by summation of 70 monolayers in the z direction.

The diffraction intensity profile of the model is obtained after Fourier transform of the periodic electron density profile calculated previously.

$$I(q) = I_e\left|\int_1^{Nd} \rho(z)e^{z\alpha}e^{iqz}dz\right|^2,$$

The $e^{z\alpha}$ coefficient represents X-ray absorption by lipid layers. This correction limits layer interference, thus the diffraction profile is smooth. The α factor is set to 0.001.

The structures which appeared in the system were described by different electron density profiles and the calculated previously intensities I for each structure were added non-coherently $I_{tot} = I_1 + I_2$.

7. Results and discussion

Fig. 1 shows X-ray scans of DPPC with addition of FABT at different temperatures. Comparison of the observed reflections around $2\theta \cong 2°$ with those from bulk crystals of FABT reveals that they come from the new periodic structures of FABT incorporated into the DPPC multibilayers (DPPC–FABT). The reflections originating from DPPC–FABT are marked with C_G and C_L in the figures. The intensities of the new reflection strongly depend on the FABT content. Reflections from the lamellar structure of the complex in the gel and liquid-crystalline phase are most clearly seen at a concentration of 10 mol% FABT (see Fig. 1 reflections C_G and C_L). For the 20 mol% FABT mixture, only the first order reflection from the complex is intense, whereas the other higher order reflections are broad. This is probably related to the