X-Ray Studies on Phospholipid Bilayers. XI. Interactions with Chloramphenicol

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Z. Naturforsch. **46c,** 647–655 (1991); received January 24, 1991

X-Ray Diffraction, Phospholipid Bilayer, Erythrocyte Membrane, Chloramphenicol

Chloramphenicol is a widely used antibiotic with low levels of toxicity. However, scanning electron microscopy revealed morphological changes in human erythrocytes when they interacted *in vitro* with therapeutical concentrations of chloramphenicol. To explain this shape change, a study concerned with the possible interactions of this antibiotic with bilayers built-up of phospholipids located in either side of the red cell membrane was performed by X-ray diffraction. Results indicated that chloramphenicol was unable to perturb in any significant extent the structure of the phospholipids under study. The only noticeable effects were phase transitions produced to dimyristoylphosphatidylethanolamine bilayers.

Introduction

It is certainly of interest to understand how therapeutical drugs and chemicals of biological interest affect the molecular structure of the erythrocyte membrane. However, this is not a simple problem given a) the large number of different proteins and lipids that form the membrane, b) the non-periodical distribution of the proteins, and c) the state of "fluidity" of the membrane. These are the main reasons that explain why, in spite of the number of studies, its detailed three-dimensional structure still remains unsolved [1, 2]. A simple, although limited, attempt to overcome this problem is the use of phospholipid bilayers. The structure of several lecithins and cephalins, phospholipids respectively located in the outer and inner side of the erythrocyte membrane, has been determined by X-ray diffraction [3, 4]. These structures correspond to those of the lipids in their crystalline phases and not to the more fluid present in the real biological membranes. However, this information and the technique are very useful for determining the degree of perturbation induced by the interaction of drugs and chemical compounds with bilayers built-up of pure lecithins and cephalins [5–8].

Chloramphenicol (CLP) is a broad-spectrum antibiotic that since its introduction in 1949 has been extensively used. Although it was thought to have no significant toxicity it was reported to produce aplastic anemia [9] and the baby syndrome [10]. As a result its use declined. However, with the appearance of ampicillin-resistant *Hemophilus influenzae,* the pathogenicity of *Bacteroides fragilis* and the conclusion that it does not present an unacceptable risk, CLP has reemerged as an important antibiotic [11, 12]. It must also be mentioned that it is one of the therapeutical drugs most used in this country [13]. CLP can be included amongst the drugs that present the physicochemical characteristic of being amphiphilic as it has a short hydrophilic chain joined to an hydrophobic ring (Fig. 1). This type of compound is able to change the shape of human erythrocytes [14, 15] and decrease its osmotic fragility [16]. The mechanism of these changes, although still poorly understood [17], has been explained by the "bilayer couple hypothesis" of Sheetz and Singer [18]. Accordingly to it, a stomatocytic shape transformation stems from a preferential intercalation of the drug in the inner hemileaflet of the lipid bilayer which tends to expand the membrane towards the inside of the cell.

This study deals with the interaction of CLP with the membrane of human erythrocytes and with model systems consisting of multibilay-

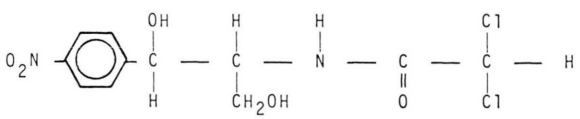

Fig. 1. Schematic structure of CLP.

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Verlag der Zeitschrift für Naturforschung, D-7400 Tübingen
0939–5075/91/0700–0647 $ 01.30/0

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ers built-up of dimyristoylphosphatidylcholine (DMPC) and of dimyristoylphosphatidylethanolamine (DMPE). These phospholipids are representative of lecithins and cephalins, which are respectively found in the outer and inner monolayers of the erythrocyte membrane [19]. They differ in their terminal amino-group, being $^{+}N(CH_3)_3$ in DMPC and $^{+}NH_3$ in DMPE. As a function of hydration and temperature these phospholipids undergo the phase transition $Lc \rightleftharpoons L\beta' \rightleftharpoons P\beta' \rightleftharpoons L\alpha$, where Lc denotes the crystalline phase, $L\beta'$ the gel phase, $P\beta'$ the rippled gel phase and $L\alpha$ the liquid-crystalline phase [20]. The bilayer structure of DMPC and DMPE are very similar in their dry crystalline phases. In fact, both have the hydrocarbon chains mostly parallel and completely extended with their polar groups lying perpendicularly to them. However, DMPE molecules pack more tightly than those of DMPC [4]. The addition of water below the main transition temperature does not significatively perturb the packing of DMPE molecules. However, the gradual hydration of DMPC under the same condition results in water occupying the highly polar interbilayer spaces, increasing their separation and the lipid undergoing a phase transition from the crystalline Lc to the lamellar $L\beta'$ [4]. These bilayer systems and methods have already been used in this laboratory to get an insight about the way chemicals of biological interest perturb the structure of cell membranes [7, 8, 21, 22].

Materials and Methods

Chemicals

Synthetic DMPE from Sigma (Lot 81F-8365), A grade, MW 677.9; DMPC from Sigma (Lots 35F-8430 and 36F-8445), A grade, MW 653.9 and CLP, a gift from Prof. R. Zemelman, Dept. Microbiology, University of Concepción, were used without further purification.

Morphological observation of the erythrocytes

Blood samples were obtained by puncture of the ear lobule, previously desinfected with 70% ethanol, from clinically healthy individuals not being treated with any pharmacological agent. The first two or three blood drops were discarded and one drop was received in a plastic tube containing 5 ml of phosphate buffered saline (PBS) (150 mM NaCl/10 mM phosphate, pH 7.4), at 5 °C. This blood stock solution (1 blood drop/5 ml PBS) was used to prepare, in plastic tubes, the following working solutions: a) 1 ml of blood stock solution plus 9 ml of PBS (control), and b) 1 ml of blood stock solution plus 9 ml of PBS containing CLP in final concentrations of 10 and 100 µg/ml. These samples were incubated at 37 °C for 1 h in an oven and then were fixed with glutaraldehyde. For this purpose, one drop of each sample was added to a tube containing 1 ml of 25% glutaraldehyde in PBS and allowed to rest for 16 h at 5 °C. The fixed samples were placed directly on aluminum stubs, air dried in an oven at 37 °C for 1 h and gold coated in a sputter coater S 150 Edwards for 3 min at 10^{-1} Torr. The final film width was 35–40 nm. The observations and photographic records were performed in an ETEC AUTOSCAN scanning electron microscope.

X-ray diffraction analysis of phospholipid bilayers

Powder mixtures of DMPC:CLP and of DMPE:CLP in 1:1 molar ratios were respectively dissolved in chloroform:methanol 3:1 and left to dry very slowly and carefully. The resulting samples, in the form of crystalline powders, were introduced in special glass capillaries of 1.5 mm diameter. They were X-ray diffracted in Debye-Scherrer cameras of 114.6 mm diameter and flat-plate cameras with 0.25 mm inner diameter glass collimators [4] provided with rotating and cooling devices. The same procedure was followed with pure samples of each phospholipid and CLP.

Hydrated samples were prepared in 2 mm diameter glass capillaries, each containing about 2 mg of recrystallized DMPC or DMPE. To each capillary, it was added about 100 µl of one of the following solvents or aqueous solutions: a) pure water; b) 5×10^{-3} M CLP; c) PBS and d) 5×10^{-3} M CLP in PBS. Samples of c) and d) were also incubated at 37 °C for 1 h and cooled down to 17 °C. All of them were X-ray diffracted in flat-plate cameras two days and two weeks after preparation. Specimen-to-film distances were either 8 or 14 cm, standardized by sprinkling calcite powder on the capillaries surfaces. Ni-filtered CuKα radiation from a Philips PW 1140 X-ray generator was used. The relative intensities of the reflections were measured from the X-ray films using a Joyce-Loebl

MK III CS microdensitometer connected to an Acer 915 microcomputer. All experiments were carried out at about 60% relative humidity and 17 °C ± 2 °C, which is below the main transition temperature of each phospholipid under study.

Results

Fig. 2 shows the scanning electron microscope images of human erythrocytes exposed *in vitro* to different concentrations of chloramphenicol. As it can be noticed, a concentration of 10 μg/ml – equivalent to that normally found in the plasma of patients under treatment with this antibiotic [13] – produced a poiquilocytosis, consisting in a deepening of the central concavity of the red cells. This shape deformation considerably increased when the CLP concentration was raised to 100 μg/ml, reaching in a few cases the shape of stomatocytes. The pores observed in the cell membrane of some erythrocytes, which reached a diameter of up to 0.3 μm, are most likely due to the fast dehydration during the drying process which produced the rupture of the cell membrane in different places.

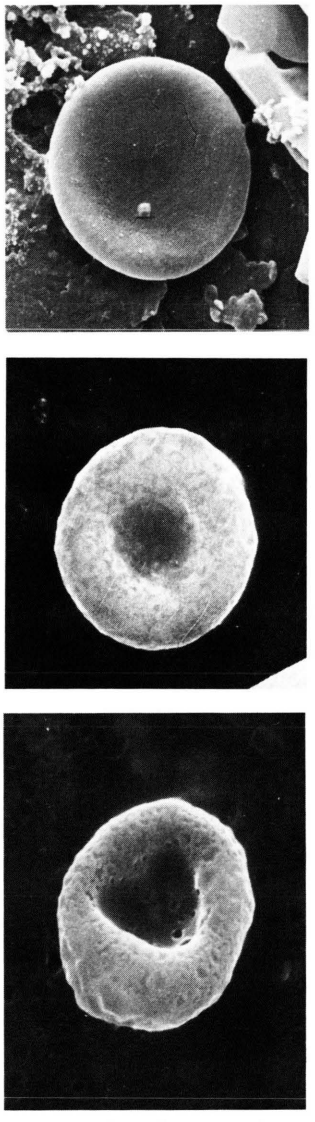

Fig. 2. Scanning electron microscope images of human erythrocytes exposed to different concentrations of CLP. (a) 0.0 μg/ml; 7200 ×. (b) 10 μg/ml; 6900 ×. (c) 100 μg/ml; 6900 ×.

In order to test whether these changes of shape were due to differential surface area expansions of either lipid monolayer in the erythrocyte membrane [18], the possible interactions of CLP with the phospholipids DMPC and DMPE were studied by X-ray diffraction techniques. The patterns were obtained from a) dry samples of CLP:phospholipid 1:1 molar mixtures recrystallized from hydrophobic solutions ($CHCl_3$:CH_3 3:1) and b) mixtures of each phospholipid in their crystalline phases with different aqueous solutions of CLP. These patterns were compared with those of the pure CLP and the corresponding phospholipid obtained under the same physicochemical conditions. The results are shown in Tables I to IV and Fig. 3 to 6. Table I presents the interplanar spacings and the relative intensities of the reflections produced by dry samples of DMPC, CLP and of their 1:1 molar mixture while their diffractograms are compared in Fig. 3. The analysis of these results indicates that all the reflections produced by the DMPC:CLP mixture belong to either the phospholipid or to CLP. In fact, their spacings are practically the same when pure or mixed within experimental error varying only their intensities as a function of their respective phase concentration. Indeed, the pattern of this mixture corresponds to a superposition of the patterns of the single crystalline phases of DMPC and CLP. On the other hand, the reflections from DMPC, either pure or mixed with CLP, indicate that it presents the bilayer structure, which has been described elsewhere [23]. This proves, unambiguously, that CLP did not chemically interact with DMPC neither intercalated in its bilayers when they were allowed to interact in an hydrophobic medium. Otherwise,

Table I. Comparison of observed interplanar spacings (do) and relative intensities (Io rel) of DMPC, DMPC:CLP 1:1 and CLP obtained from dry powder samples recrystallized from $CHCl_3$:CH_3OH 3:1 (a), (b).

DMPC		DMPC:CLP 1:1		CLP	
do [Å]	Io rel	do [Å]	Io rel	do [Å]	Io rel
55.2*	460	55.2*	204	–	–
27.8	19	27.6	7	–	–
18.5	9	18.5	2	–	–
16.1	1	–	–	–	–
13.7	24	13.7	6	–	–
–	–	11.0	3	11.10	6
9.27	11	9.29	4	–	–
8.67	2	–	–	–	–
8.28	2	–	–	–	–
–	–	8.16	1	8.14	3
7.28	2	–	–	–	–
–	–	6.84	6	6.87	14
6.31	15	6.32	11	–	–
5.84	1	–	–	–	–
–	–	5.63	9	5.63	14
5.28	5	5.25	2	–	–
–	–	4.98	12	4.98	17
4.91	2	–	–	–	–
4.67	9	4.67	12	4.68	15
–	–	4.48	2	4.47	11
4.32	39	4.29	51	4.27	30
4.09	57	4.10	47	–	–
–	–	–	–	3.92	3
3.84	9	3.83	10	3.86	3
3.71	2	–	–	–	–
–	–	3.66	28	3.66	36
–	–	3.44	4	3.42	6
–	–	3.35	3	3.34	2
3.19	5	3.22	3	3.23	9
–	–	–	–	3.13	2
3.05	1	3.04	5	3.04	6

(a) The interplanar spacings and intensities of the reflections were measured in X-ray diagrams obtained from flat-plate cameras. D = 8 and 14 cm*.
(b) Several additional reflections with spacings below 3.0 Å were also observed.

the X-ray pattern of DMPC in the mixture would have differed from that obtained from its single phase.

Table II and Fig. 4 show the results obtained from dry samples of DMPE, CLP and of their 1:1 molar mixture. It has been found that this phospholipid presents two polymorphic forms when it is recrystallized from $CHCl_3$:CH_3OH solutions

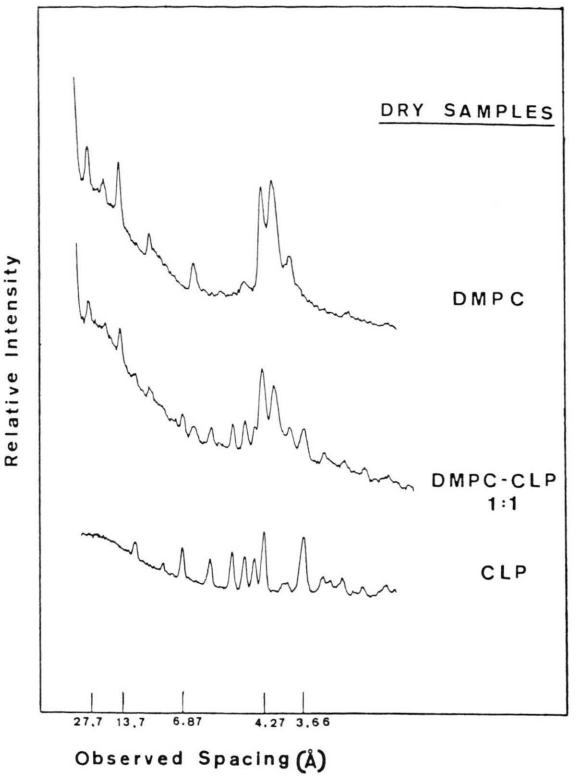

Fig. 3. Microddensitograms from X-ray diffraction diagrams of dry specimens recrystallized from $CHCl_3$:CH_3OH 3:1. Flat-plate cameras (D = 8 cm).

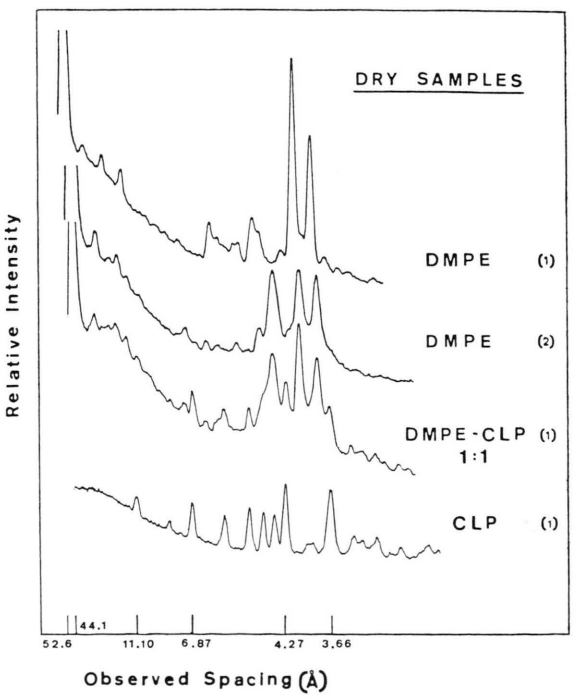

Fig. 4. Microdensitograms from X-ray diffraction diagrams of dry specimens recrystallized from (1) $CHCl_3$:CH_3OH 3:1 and (2) $CHCl_3$:CH_3OH 1:3. Flat-plate cameras (D = 8 cm).

Table II. Comparison of observed interplanar spacings (do) and relative intensities (Io rel) of dry powder samples of DMPE recrystallized from $CHCl_3$:CH_3OH 1:3, and of DMPE, DMPE:CLP 1:1 and CLP recrystallized from $CHCl_3$:CH_3OH 3:1 (a), (b).

DMPE (1)		DMPE (2)		DMPC:CLP 1:1 (1)		CLP (1)	
do [Å]	Io rel	do [Å]	Io rel	do [Å]	Io rel	do [Å]	Io rel
52.6*	140	–	–	–	–	–	–
–	–	44.1	360	44.0	780	–	–
26.1	7	–	–	–	–	–	–
–	–	21.7	9	22.0	7	–	–
17.5	9	–	–	–	–	–	–
–	–	16.9	3	16.9	3	–	–
–	–	14.7	7	14.8	4	–	–
13.15	8	–	–	–	–	–	–
–	–	12.7	2	12.7	4	–	–
–	–	–	–	11.2	5	11.10	6
–	–	–	–	–	–	8.14	3
–	–	7.25	4	7.30	5	–	–
–	–	–	–	6.87	10	6.87	14
–	–	6.30	5	6.34	4	–	–
6.13	17	–	–	–	–	–	–
–	–	5.95	6	5.94	4	–	–
5.88	12	–	–	–	–	–	–
5.62	7	–	–	5.63	6	5.63	14
5.37	19	5.30	7	5.32	3	–	–
5.22	10	–	–	–	–	–	–
–	–	–	–	5.03	8	4.98	17
4.92	26	–	–	–	–	–	–
4.76	16	4.76	7	–	–	–	–
–	–	–	–	4.65	13	4.68	15
–	–	4.47	60	4.47	58	4.47	11
4.36	4	–	–	–	–	–	–
–	–	–	–	4.27	18	4.27	30
4.15	4	4.19	2	–	–	–	–
4.02	110	4.03	60	4.05	49	–	–
3.90	54	–	–	–	–	3.92	3
–	–	–	–	–	–	3.86	3
–	–	3.80	50	3.80	31	–	–
3.73	5	–	–	–	–	–	–
3.53	2	3.50	2	–	–	–	–
3.48	2	–	–	–	–	–	–
–	–	–	–	3.42	9	3.42	6
–	–	–	–	3.34	1	3.34	2
3.24	1	–	–	3.22	5	3.23	9
–	–	3.12	1	–	–	3.13	2
3.04	6	–	–	3.05	2	3.04	6

(a) The interplanar spacings and intensities of the reflections were measured in X-ray diagrams obtained from flat-plate cameras. D = 9 and 14 cm*.
(b) Several additional reflections with spacings below 3.0 Å were also observed.
(1) Recrystallized from $CHCl_3$:CH_3OH 3:1.
(2) Recrystallized from $CHCl_3$:CH_3OH 1:3.

[22]. One phase (Lc_1) is obtained when these solvents are in a 3:1 v/v ratio; its bilayers repeat of 52.6 Å indicates that the hydrocarbon chains are extended and parallel to the bilayer normal [24]. The other phase (Lc_2) of DMPE can be obtained by its recrystallization from $CHCl_3$:CH_3OH 1:3. In this case, the bilayer repeat is 44.1 Å because the hydrocarbon chains are now tilted by about 33°. The observed pattern of the DMPE:CLP 1:1 mixture corresponds to the superposition of the pattern of CLP and that of the Lc_2 phase of DMPE, in spite of the fact the mixture was recrystallized from $CHCl_3$:CH_3OH 3:1. This was the only noticeable change that CLP produced to the bilayer

structure of DMPE under the above described conditions.

Fig. 5 and Table II show some of the X-ray patterns and their interplanar spacings obtained two days and two weeks after DMPC in its crystalline phase was mixed and allowed to interact with a) water, b) 5×10^{-3} M CLP aqueous solution, c) 5×10^{-3} M CLP in PBS, and d) PBS, with and without incubation at 37 °C for 1 h. These experiments were designed to cover most of the physicochemical conditions under which CLP interacted with the human erythrocytes and produced the observed shape changes. However, the CLP 5×10^{-3} M concentration in the X-ray experiments (1.6 mg/ml) was much higher than those used with the red blood cells (10 and 100 μg/ml). The results obtained indicate that CLP, in spite of its high concentration, produced only slight perturbations on DMPC phospholipid bilayers.

In both Fig. 5 and Table III it is possible to appreciate that pure water expanded DMPC bilayer period from 55.4 Å when dry to 65.2 Å. At the same time, DMPC changed from the crystalline Lc phase to the lamellar Lβ′. The observed reflections of the latter were reduced to only the first three orders of the 65.2 Å repeat in the low angle region and one of 4.2 Å in the high angle. This reflection is present in lecithin:water mixtures below their main transition temperatures (β and β′ phases) and it arises from the stiff and fully extended hydrocarbon chains organized with rotational disorder in an hexagonal lattice [20]. As Fig. 5 and Table III show, this pattern of DMPC did not significatively change under the single and combined effects of chloramphenicol, buffer, incubation and time.

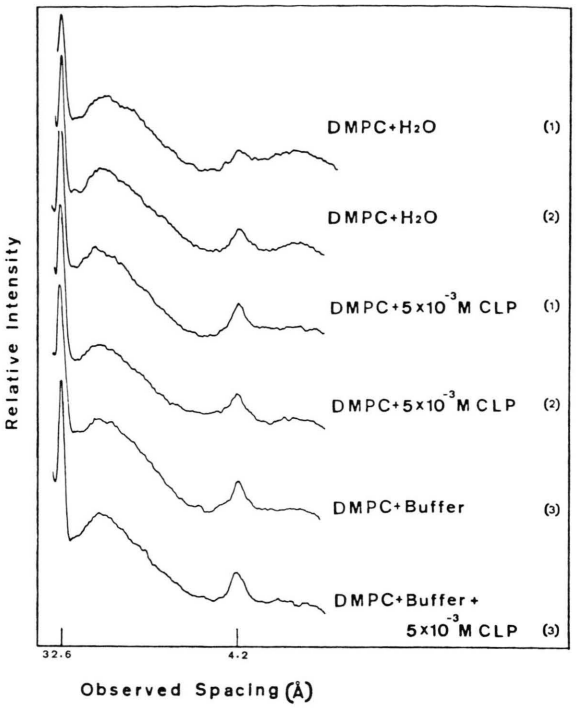

Fig. 5. Microdensitograms from X-ray diffraction diagrams of DMPC in a hydrophilic medium. (1) Two days after preparation; (2) fourteen days after preparation; (3) after incubation at 37 °C for 1 h. Flat-plate cameras (D = 8 cm).

About the same results were obtained in DMPE as it can be observed in Fig. 6 and Table IV. In fact, the X-ray patterns of DMPE did not change

Table III. Comparison of observed interplanar spacings (do) and relative intensities (Io rel) of DMPC powder samples mixed with water and CLP aqueous solutions (a), (b).

DMPC+H_2O in excess (1)		DMPC+H_2O in excess (2)		DMPC+5×10^{-3} M CLP (1)		DMPC+5×10^{-3} M CLP (2)		DMPC+Buffer (3)		DMPC+Buffer+ 5×10^{-3} M CLP (3)	
do [Å]	Io rel	do [Å]	Io rel	do [Å]	Io rel	do [Å]	Io rel	do [Å]	Io rel	do [Å]	Io rel
65*	19	65*	72	65*	300	65*	400	65*	122	65*	129
33.0	29	32.5	84	32.7	170	33.0	231	32.6	122	32.6	129
21.2	2	21.2	4	–	–	–	–	21.3	3	21.0	2
13.9	46	13.8	68	14.0	20	13.8	17	13.8	56	13.8	80
4.19	38	4.18	26	4.20	62	4.19	46	4.20	31	4.20	48

(a) Additional X-ray diagrams of all these samples were taken with and without incubation at 37 °C for 1 h. They did not show relevant differences with the data included in this Table.
(b) The interplanar spacings and intensities of the reflections were measured in X-ray diagrams obtained from flat-plate cameras. D = 8 and 14 cm*.
(1) The samples were left for 2 days before being X-ray diffracted.
(2) The samples were X-ray diffracted 12 days after (1).
(3) The samples were incubated at 37 °C for 1 h before being X-ray diffracted.

in a significant extent under the different conditions its samples were assayed. The only difference with respect to DMPC was that DMPE remained in the crystalline Lc_1 phase, very similar to that observed in the dry state. The only variation was observed 48 h after one sample of DMPE was immersed in 5×10^{-3} M CLP, when it showed the Lc_2 phase. However, two weeks later the same sample exhibited again the Lc_1 phase.

Discussion

Given the widespread use of chloramphenicol and its amphiphilic character, it was thought of interest to study the possible perturbation that it could produce to cell membranes. For this purpose, human erythrocytes were made to interact *in vitro* with CLP in a concentration equivalent to that found in the plasma when it is therapeutically administered. Observations made by electron microscopy revealed that CLP produced a marked cell deformation which increased with concentration. This type of shape change has been explained as due to a differential expansion of either monolayer of the erythrocyte membrane in the plane of the membrane as a result of their different interactions with amphiphilic drugs [18]. This study, therefore, was carried out to test whether the erythrocyte shape deformation was indeed due to different types of interactions of CLP with phospholipids located in each side of the membrane bilayer. For this purpose, DMPE and DMPC were used as they represent phospholipids which are respectively found in the inner and outer halves of the human red cell membranes. Their interactions with CLP were studied by X-ray diffraction under two different physicochemical conditions. In the first, CLP was allowed to interact with each phospholipid in an hydrophobic medium ($CHCl_3$:CH_3OH 3:1 solution) for about 24 h, after which the solvent was slowly removed by evaporation at about 17 °C.

Table IV. Comparison of observed interplanar spacings (do) and relative intensities (Io rel) of DMPE powder samples mixed with water and CLP aqueous solutions (a), (b).

DMPC+H_2O in excess (1)		DMPE+H_2O in excess (2)		DMPE+5×10^{-3} M CLP (1)		DMPE+5×10^{-3} M CLP (2)		DMPE+Buffer (3)		DMPE+Buffer+5×10^{-3} M CLP (3)	
do [Å]	Io rel	do [Å]	Io rel	do [Å]	Io rel	do [Å]	Io rel	do [Å]	Io rel	do [Å]	Io rel
51.3*	250	51.3*	233	–	–	51.3*	240	51.3*	188	51.3*	100
–	–	–	–	44.0	290	–	–	–	–	–	–
25.2	1	25.2	1	–	–	25.3	3	–	–	25.2	2
–	–	–	–	21.7	3	–	–	–	–	–	–
17.1	6	16.9	3	–	–	16.9	4	17.1	4	17.1	7
–	–	–	–	14.6	2	–	–	–	–	–	–
12.8	10	13.0	5	–	–	12.9	8	12.8	5	12.8	8
7.34	3	7.35	1	–	–	7.33	1	7.30	1	7.31	2
6.00	10	6.00	1	–	–	6.02	7	5.98	4	6.02	6
5.09	1	5.08	5	–	–	5.10	3	5.11	3	5.11	2
4.82	11	4.84	2	–	–	4.82	8	4.80	8	4.81	14
4.66	1	–	–	–	–	4.66	4	4.66	2	4.68	5
4.52	1	4.51	3	–	–	–	–	–	–	–	–
–	–	–	–	4.47	17	–	–	–	–	–	–
4.23	2	4.26	1	–	–	4.26	1	4.26	1	4.23	3
4.06	63	4.08	43	4.06	11	4.07	43	4.06	32	4.06	50
3.81	30	3.83	19	3.80	7	3.82	20	3.81	13	3.81	29
3.65	2	3.67	2	–	–	3.67	1	3.65	1	3.67	2
3.52	2	–	–	–	–	–	–	–	–	3.52	2
3.41	2	–	–	–	–	–	–	–	–	3.41	2
3.18	1	–	–	–	–	–	–	3.19	2	3.19	3

(a) Additional X-ray diagrams of all these samples were taken with and without incubation at 37 °C for 1 h. They did not show relevant differences with the data included in this Table.
(b) The interplanar spacings and intensites of the reflections were measured in X-ray diagrams obtained from flat-plate cameras. D = 8 and 14 cm*.
(1) The specimens were left for 2 days before being X-ray diffracted.
(2) The specimens were X-ray diffracted 12 days after (1).
(3) The specimens were incubated at 37 °C for 1 h before being X-ray diffracted.

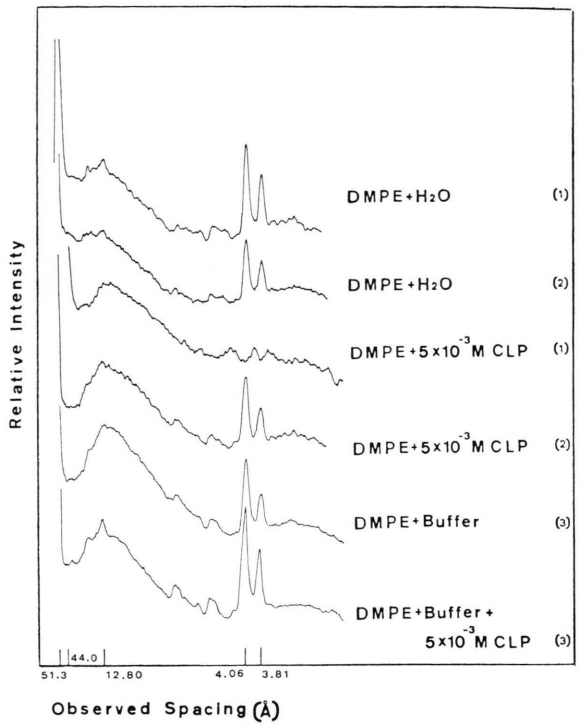

Fig. 6. Microdensitograms from X-ray diffraction diagrams of DMPE in a hydrophilic medium. (1) Two days after preparation; (2) fourteen days after preparation; (3) after incubation at 37 °C for 1 h. Flat-plate cameras (D = 8 cm).

Each CLP:phospholipid 1:1 molar mixture, under the form of dry and crystalline powders, were X-ray diffracted. The comparison of their patterns with those of the single phases of CLP, DMPE and DMPC recrystallized and diffracted under the same conditions as their respective mixtures proved that no significant interaction occurred. It was only observed that DMPE, in the presence of CLP, recrystallized in the Lc_2 phase instead of the Lc_1, which is the phase under which DMPE recrystallizes from $CHCl_3$:CH_3OH 3:1.

The second type of conditions were intented to be as close as possible to those under which CLP produced the erythrocyte shape change and they included CLP concentration, buffer, temperature and time of incubation. As no structural perturbations were observed in any of the phospholipid bilayers, CLP concentration and the incubation time at 17 °C were considerably increased. In spite of these extreme conditions, the only noticeable effect was again a phase transition of DMPE from Lc_1 to Lc_2, which reversed to the Lc_1 phase ten days later.

These results clearly indicate that CLP did not affect in any significant extent the bilayer structure of the phospholipids under study. This conclusion is rather surprising as another amphiphilic antibiotic, chlortetracycline hydrochloride, was able to produce deep perturbations to DMPC both in a hydrophobic and hydrophilic medium at lower concentrations and milder conditions than those that were used with CLP [22]. It is, therefore, possible to attribute to CLP interactions with proteins present in the erythrocyte membrane the cell deformation observed *in vitro*.

Finally, a few words must be said about the different phase transitions observed in DMPC and DMPE. The former changed from the Lc phase to the Lβ′ simply as a function of hydration. As explained in the introduction, this effect is due to the large and highly polar interbilayer spaces present in the Lc phase. As water fills in, the bilayer repeat increases from 55.4 Å to 65.2 Å, the DMPC molecules become more disordered and eventually they rearrange in a hexagonal packing [20]. On the contrary, DMPE molecules are so tightly packed in the crystalline phase that water is unable to perturb their arrangement in any significant degree.

Although CLP did not produce any change on DMPC, it was able to induce to DMPE a transition from the Lc_1 phase to the inclined Lc_2, both in hydrophobic and hydrophilic media. This effect was observed in aqueous solutions only when DMPE, in the Lc_1 phase, was mixed with 5×10^{-3} M CLP. However, ten days later DMPE showed again the Lc_1 phase. The explanation for the reversibility of the phase transition lies in that the physicochemical conditions of the CLP solution were such that favored the tilted phase of DMPE. Nevertheless, this phase could not remain for too long due to the unstability of CLP in aqueous solutions in which undergoes a variety of hydrolitic and light-induced reactions [25]. A similar effect on DMPE has been previously reported for chlortetracycline hydrochloride [22], which is also unstable in aqueous solutions [26].

Acknowledgements

Research grants from FONDECYT (0783/88) and from the University of Concepción (20.13.79) are gratefully acknowledged.

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