ELSEVIER

Biophysical Chemistry 111 (2004) 1–7

Biophysical Chemistry

www.elsevier.com/locate/bpc

Preliminary studies of the 2D crystallization of Omp1 of *Serratia marcescens*: observation by atomic force microscopy in native membranes environment and reconstituted in proteolipid sheets

Neus Ruiz[a], Sandra Merino[b,c], Miquel Viñas[a], Òscar Domènech[c,d], M. Teresa Montero[b,c], Jordi Hernández-Borrell[b,c,*]

[a] *Laboratori de Microbiologia Campus de Bellvitge, Universitat de Barcelona E-08907-L'Hospitalet de Llobregat, Spain*
[b] *Departament de Fisicoquímica, Universitat de Barcelona E-08028-Barcelona, Spain*
[c] *Centre de Bioelectrònica i Nanobiociència (CBEN), Parc Científic de Barcelona, Spain*
[d] *Química-Física, Universitat de Barcelona E-08028-Barcelona, Spain*

Received 25 February 2004; received in revised form 25 February 2004; accepted 5 March 2004
Available online 6 May 2004

Abstract

In this work the porin Omp1 of *Serratia marcescens* was expressed in a porin deficient mutant (*Escherichia coli* UH302) and its functionality studied following the accumulation of ciprofloxacin in bacteria. The protein was extracted, purified and reconstituted in proteoliposomes of different composition (lipopolysaccharide (LPS), 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC) and, 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC)). Maximum extraction of the detergent was achieved applying different steps of dialysis and centrifugation. Proteolipid sheets with different composition were spread onto mica and observed by atomic force microscopy. Two-dimensional crystal of Omp1 was not observed in any case due to low resolution achieved. Judging from the images features POPC is the most suitable phospholipid to enhance 2D lattice formation for Omp1.

Keywords: 2D crystallization; Omp1; *Serratia marcescens*; Atomic force microscopy

1. Introduction

The outer membrane of Gram-negative bacteria constitutes the biointerface between bacteria and the surrounding medium. The outer membrane is formed by lipid bilayer regions, consisting of proteins, phospholipids, or lipopolysaccharide (LPS) molecules. Unusually, this barrier shows low permeability towards hydrophobic solutes as it has been demonstrated in different microorganisms using i.e. a homologous series of antibiotics [1]. The outer membrane opposes, however, resistance to most hydrophilic molecules that cross the membrane through a few major transmembrane proteins called ''porins'' [2]. Porins are β-sheet channel-forming proteins [3] many of which are either non-specific, such as OmpF or OmpC, or only moderately selective, such as PhoE in *Escherichia coli.*

Serratia marcescens an enterobacterial species that produces a wide range of opportunistic infections and can cause nosocomial outbreaks has been the focus of our research for many years [4–7]. *S. marcescens* outer membrane contains three different general diffusion porins: Omp1, Omp2 and Omp3, with apparent molecular weights of 42, 40 and 39 kDa, respectively [5,8]. *Omp1* was cloned, its nucleotide sequence determined and expressed in a porin deficient strain, *E. coli* UH302 (*E. coli* UH302 pOM100). Protein Omp1 has been thereafter purified to homogeneity reconstituted into black lipid bilayers and its channel-forming activity conclusively demonstrated [9]. The porin appears to be a homotrimeric protein formed by three identical polypeptide subunits of 375 amino acids. Parallel to the conventional 3D crystallization, when the final amount of the purified membrane protein is little (1–2 mg), such is the

* Corresponding author. Departament de Fisicoquímica, Facultat de Farmàcia, U.B. 08028-Barcelona, Spain. Tel.: +34-93-403-59-86; fax: +34-93-403-59-87.
E-mail address: jordihernandezborrell@ub.edu (J. Hernández-Borrell).

0301-4622/$ - see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.bpc.2004.03.006

case, other strategies could be considered. Among them crystallization in two-dimensions (2D) [10] or in cubo crystallization [11] have been successfully applied in many cases. To obtain 2D membrane protein crystals, proteolipid sheets (PLSs) were obtained from protein reconstituted in lipid bilayers, normally spread onto an adequate surface (i.e. mica) and observed generally by electron microscopy and more recently by atomic force microscopy (AFM).

The present paper has the following objectives: (i) to demonstrate the expression and activity of Omp1 following the accumulation of a 6-fluoroquinolone antibiotic (6-ciprofloxacin) into the *E. coli* UH302 and *E. coli* UH302 pOM100 cells; (ii) to investigate if ubiquous crystallization of the protein occur by observation in situ of outer membranes extracted from the recombinant *E. coli* UH302 pOM100; and (iii) to select the adequate phospholipid composition to obtain 2D crystals from PLSs. This preliminary work becomes necessary because the phospholipid matrix might eventually determine the protein packing [12]. Besides, it compromise future structural studies on the 2D crystallized membrane protein.

2. Materials and methods

2.1. Chemicals

1-Palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC), 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC), octyl glucoside (OG), and lipopolysaccharide from *E. coli* serotype Oss:B5 were purchased from Sigma, St. Louis, MO, USA. Genapol was from Fluka, Spain. All other common chemicals were ACS grade.

2.2. Bacterial strains and growth conditions

S. marcescens 2170 is an environmental isolate [13] and *E. coli* UH302 [14], a porin-deficient strain, was used for cloning and expression experiments. Strains were grown in Trypticase Soy Broth (TSB) for outer membrane protein preparations and porin purification, purchased from Liofilchem Bacteriological Products (Italy). Ciprofloxacin (Cip) was kindly supplied by CENAVISA Laboratories (Reus, Spain).

2.3. Expression and purification of the cloned porin in E. coli UH302

The *S. marcescens* Omp1 porin was expressed in *E. coli* UH302. The Omp1 protein was purified as follows: bacteria were growth overnight in TSB supplemented with ampicillin 100 μg/ml and harvested by centrifugation (1000×*g*, 10 min), washed once with a 10 mM Tris–HCl pH 7.4 and resuspended in the same buffer. Cells were broken by ultrasonic treatment and unbroken cells removed by centrifugation (1000×*g*, 10 min). The supernatant was centrifuged (100,000×*g* for 1 h), sedimented bacterial envelopes were resuspended in a buffer containing 2% SDS, 10 mM Tris–HCl pH 7.4. The peptidoglycan layer and the associated proteins were pelleted by centrifugation at 100,000×*g* for 30 min. The pellet was subjected to a second SDS wash. The final pellet was suspended in a buffer containing 2% Genapol, 10 mM Tris–HCl pH 7.4 and 2 mM ethylenediamine tetraacetic acid (EDTA). The supernatant of the subsequent centrifugation (100,000×*g*, 30 min) contained pure Omp1 porin. To visualize Omp fractions, SDS-PAGE was performed in a Bio-Rad apparatus (miniprotean II) gels were stained with 0.25% Coomassie brilliant blue, destained and finally dried using a gel dryer (BioRad 543).

2.4. Determination of fluoroquinolone accumulation

Fluoroquinolone accumulation was followed using a procedure elsewhere described [6] which takes the advantage of the intrinsic fluorescence of the antibiotic [15]. Isolates were incubated at 37 °C until A_{600nm}=0.5–0.7. Bacteria were harvested by centrifugation (9000×*g*) at room temperature, washed and concentrated 10-fold in phosphate buffer saline (PBS) pH 7.5. Fluoroquinolone was added to 1-ml aliquots to a final concentration of 10 μg/ml. At time intervals of 0.25, 0.5, 1.5, 3, 6, 8, 10, 15 and 20 min, samples were centrifuged in a microfuge at 10,000 rpm at 4 °C for 1 min. Pellets were resuspended in 1 ml of 0.1 M glycine–HCl buffer at pH 3.0, and finally incubated at room temperature overnight to allow bacterial lysis. Thereafter, the suspensions were centrifuged at 20 °C for 25 min to remove bacterial debris.

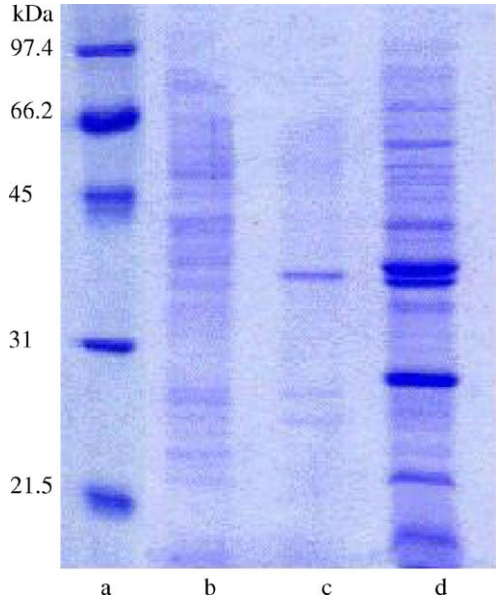

Fig. 1. SDS-PAGE of outer membrane proteins. Standard molecular weight (a), *E. coli* UH302 (b), *E. coli* UH302 pOM100 (c) and *S. marcescens* (d). Omp1 porin is expressed in recombinant strain.

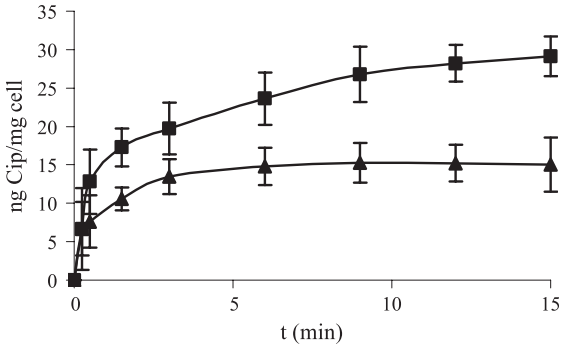

Fig. 2. Ciprofloxacin accumulation of *E. coli* UH302 (▲) and *E. coli* UH302 pOM100. (■) The values are the average of three independent measurements.

The concentration of antibiotic in the supernatants was determined fluorometrically using an SLM Aminco 8100 spectrofluorometer.

2.5. Membrane preparation and protein reconstitution

For protein reconstitution a chloroform/methanol (50:50, v/v) solution of POPC was dried under a stream of oxygen-free N_2 in a conical tube and the thin film obtained kept under high vacuum for approx. 3 h to remove organic solvent traces. Liposomes were obtained by redispersion of the lipid film in 10 mM Tris–HCl, 150 mM NaCl buffer (pH 7.40) applying successive cycles of freezing and thawing, below and above the phase transition of the phospholipid. Thereafter liposomes, supplemented with 2% (w/v) of OG, were sonicated for 30 min in a bath. Purified Omp1 solubilized in OG were mixed with the liposomes to obtain a lipid-to-protein ratio (LPR) (w/w) lower than 1 to a total protein concentration of 100 μM. Several steps have been followed to extract the detergent: (i) incubation of the mixture at 4 °C for 30 min; (ii) incorporation of the sample into dialysis cassette (Slide-A-Lyzer®) in presence of dialysis buffer (10 mM Tris–HCl, 150 mM NaCl pH 7.40); (iii)

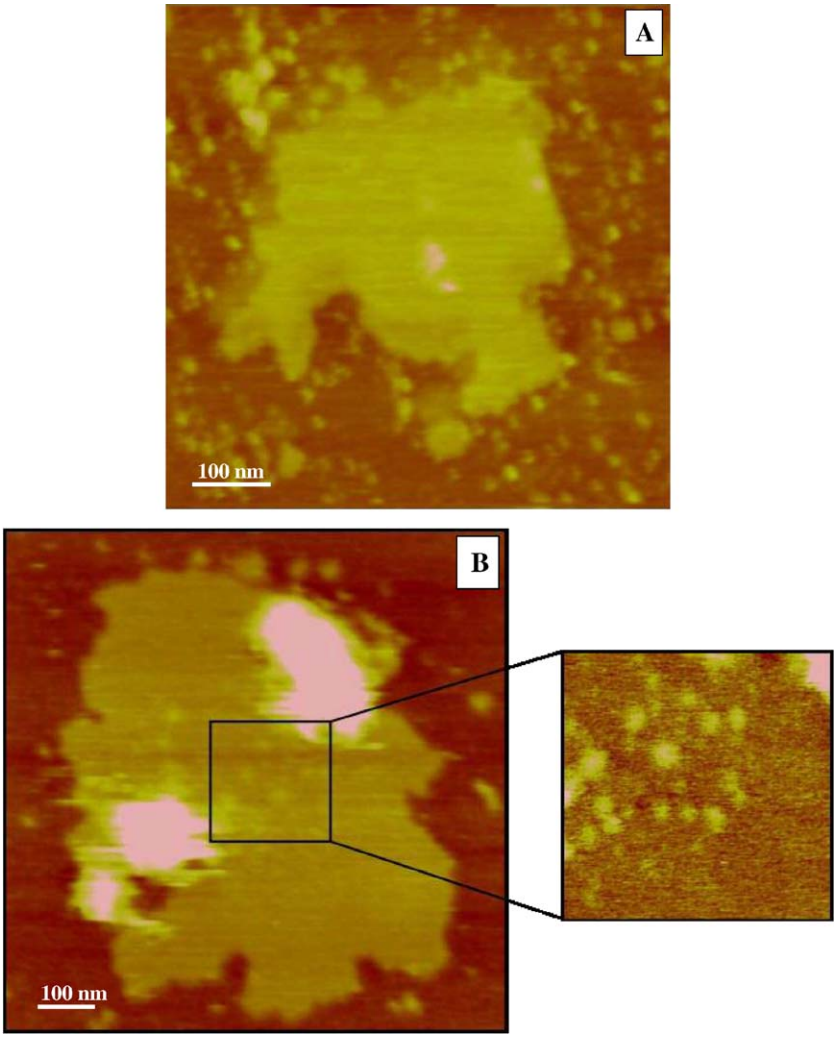

Fig. 3. Atomic force image of topography (tapping mode) of the membrane extracted from *E. coli* UH302 (A) and *E.coli* UH302 pOM100 (B) in 10 mM Tris–HCl pH 7.40, 150 mM NaCl. Zoom of surface bilayer to appreciate a detail of protrusions.

dialysis of the sample during 3 days at room temperature changing the dialysis buffer every 12 hours; (iv) centrifugation at 12,000 r.p.m. for 1 h; (v) resuspension of the pellet to a final concentration 100 μM in buffer 20 mM Hepes, 300 mM KCl, 25 mM $MgCl_2$ pH 6.40; and (vi) stabilization of the sample overnight at room temperature.

2.6. AFM observations

Experiments were carried out as in previous works [16]. Images were recorded in contact or tapping mode with a commercial Digital Instruments (Santa Barbara, CA) Nanoscope III AFM fitted with a 15-μm scanner (d-scanner). Standard Si_3N_4 tips, with a nominal force constant of 0.12 $N \cdot m^{-1}$ (Digital Instruments), were used and the forces exerted by the tip were minimized by previously recording force plots to each sample. Images were obtained in situ using an AFM fitted with a tapping or contact-mode liquid cell. Before every sample, the AFM liquid cell was washed with ethanol and ultra pure water (Milli Q reverse osmosis system), and allowed to dry in a N_2 stream. Mica discs (green muscovite mica) were cleaved with scotch and glued onto a Teflon disc by a water-insoluble epoxy. These Teflon discs were glued onto a steel disc and then mounted onto the piezoelectric scanner. Previous to imaging the sample, the tip-sample pair was thermally stabilized. Then, aliquots of 50 μl of reconstituted Omp1 in proteoliposomes samples were pipetted onto freshly cleaved mica, allowing the sample to stabilize at 25 °C (above the transition temperature of the phospholipid mixture) for 30 min (approx.) and thereafter rinsing the surface with divalent cations free. The tip was immediately immersed in the buffer. To accomplish all these experiments it was necessary to drift equilibrate the cantilever for 30 min before imaging.

3. Results and discussion

Omp1 has been described as a porin throughout small hydrophilic compounds such as some β-lactams, aminoglycosides, tetracycline and chloramphenicol penetrate the cells [6,8]. Therefore its absence confers high level of resistance against these antibiotics. The *S. marcescens* Omp1 protein was effectively expressed in *E. coli* UH302, a well known porin-deficient strain, as can be seen in the SDS-PAGE gel shown in Fig. 1. This clone (*E. coli* UH302 pOM100) was used (i) to determine the functionality of the protein; and (ii) to purify Omp1 for the biophysical experiments.

Whereas the lack of porins in *E. coli* UH302 results in an extremely low growth rate, the clone grew up much more rapidly (data not shown). As in other papers [17] accumulation experiments of ciprofloxacin into bacteria were used to determine the role of Omp1 in the uptake process. Conclusively these experiments demonstrate that, ciprofloxacin uses Omp1 to penetrate *Serratia* outer surface (Fig. 2)[1]. As can be seen, the expression of Omp1 porin in *E. coli* UH302 leads to an increase in ciprofloxacin accumulation when compared with the parental strain.

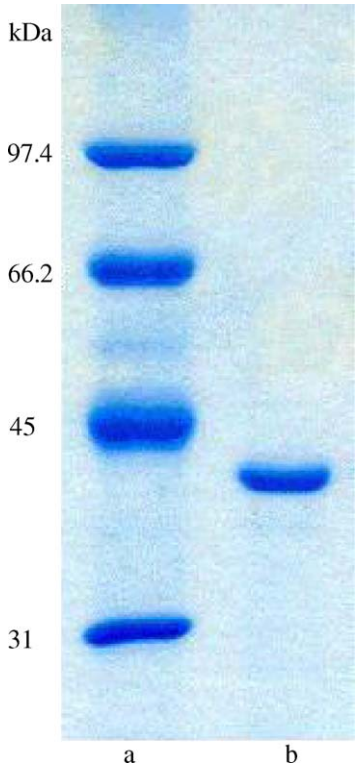

Fig. 4. SDS-PAGE of the Omp1 purification protein from *E. coli* UH302 pOM100. Standard molecular weight (a), Omp1 purified and heated previously (b).

The outer membranes isolated form *E. coli* UH302 and *E. coli* UH302 pOM100 were resuspended in buffer and imaged by AFM (Fig. 3A and B, respectively). The images were taken in tapping mode. Both membranes shown a similar height of 3.72±0.06 nm (n=10) and 3.68±0.12 nm (n=10), respectively, as measured at the edge of the proteolipid sheets. This is consistent with the values reported for other proteolipid sheets [18–20]. While the *E. coli* UH302 outer membranes were flat (Fig. 3A) some protrusions appear at the top of the *E. coli* UH302 pOM100 outer membrane extracts (Fig. 3B). Those protrusions (see Zoom of Fig. 3B) exceed 1.0±0.2 nm (n=20) from the background layer and show an average diameter of 18±3 nm (n=20). This size is higher than the one predicted from our theoretical model [9] and also higher than the values reported for crystals of other porins (i.e. [18,21–23]). However, except

[1] Ciprofloxacin is a fluoroquinolone that can use three different pathways to penetrate the bacteria [7]: (i) an hydrophobic way throughout the lipid bilayer; (ii) a self-promoted still not well known; and (iii) a hydrophilic way due to the channel forming activity of porins. If one of these pathways of entry was not functional (*E. coli* UH302) the ciprofloxacin permeation should be slower.

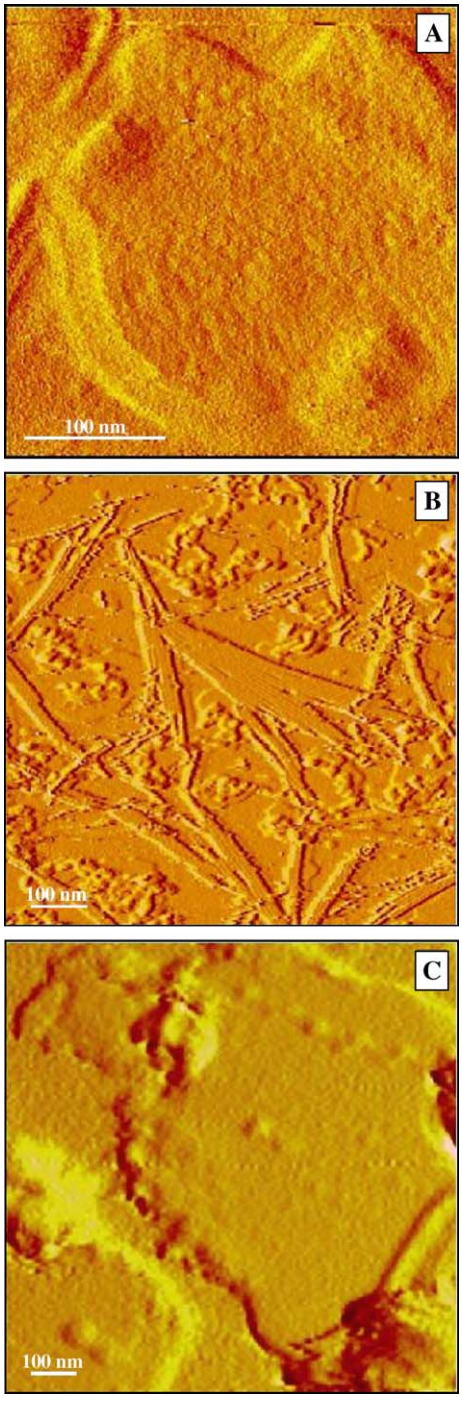

Fig. 5. Atomic force image of amplitude (tapping mode) of Omp1 reconstituted in different lipid matrix. (A) LPS. (B) DMPC. (C) DMPC:POPC (1:1) (mol/mol). Imaging buffer: 20 mM Hepes pH 6.4, 100 mM KCl.

for the purple membrane [24], the cardiac gap junction channels [25] and few more examples in situ crystallization in native membranes is uncommon. On the other hand the protrusions in Fig. 3B could easily result from the self-aggregation of the porin, probably as a trimer. Besides, there is a typical overestimation of the size (about 10%) due to the convolution of the tip [26]. Then introducing this correction [27] the diameter of the protrusions become 5.5±0.9 nm, much more in agreement with the values expected for a porin trimer.

In the reconstitution experiments the purity of the membrane protein is of crucial importance. Therefore we show in Fig. 4 an example of a SDS-PAGE gel of the purified Omp1 protein that has been used in the following experiments. Other variables as pH, ionic strength and several phospholipidic matrices were screened to obtain the best conditions for reconstitution in a biomimetic environment.

In Fig. 5 we show different kind of structures of some of the PLS obtained with different lipid matrices. In Fig. 5A a double layered PLS in a matrix of lipopolysacharide of *E. coli* is shown. These proteolipid sheets showed a rough surface due to the incorporation of the porin into the layer. However no ordered pattern was observed. On the other hand this matrix it was not possible to flat this PLS on the mica. This roughness becomes a serious difficulty during the scanning of the surface and not allows the correct visualization of a pattern under those experimental conditions.

Two pure phospholipids, POPC and DMPC, with transition temperatures of −2.5 and 24 °C, respectively, were used because they cover the range of temperatures reached during the reconstitution process (see Methods). The Fig. 5B shows the proteolipid sheets formed with pure DMPC as the matrix. The structures observed were tubular-like, showing several states of aggregation. These structures have been observed in other reconstitution experiments [28] and result apparently from the formation of tubular micelles or tubular 2D crystals. Consequently, DMPC was also discarded as lipid matrix for Omp1.

In Fig. 5C an equimolar mixture of DMPC and POPC was used as a matrix. Transition temperature of this mixture ~13 °C was calculated by fluorescence polarization (result not shown). Interestingly, after the incorporation of the porin

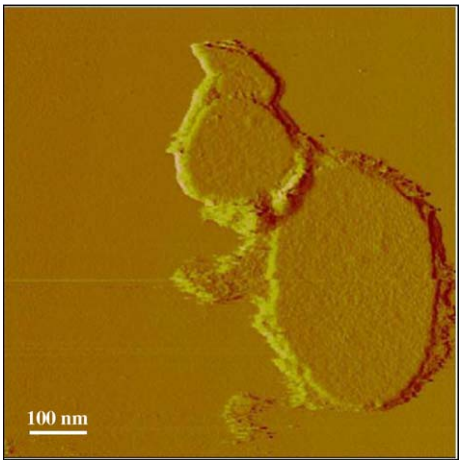

Fig. 6. Atomic force image of deflection (contact mode) of Omp1 reconstituted in POPC at LPR of 0.5. Imaging buffer: 20 mM Hepes pH 6.4, 100 mM KCl.

into the bilayer its fluidity remains constant. Proteolipid sheets formed with DMPC:POPC (Fig. 5C) showed the structure of typical bilayer obtained after spreading. Although no conclusive, certain structures on the top of the bilayer surface, similar to those observed in the natural extract of *E. coli* (Fig. 3B), were observed.

Judging only the quality of the images, the best proteolipid sheets were obtained using pure POPC (Fig. 6). These PLSs are similar to others reported in the literature and obtained with other proteins [18,19]. The PLSs were 10.0 ± 0.3 nm (n=10) height, as measured at the edges and can be interpreted as a double layered structure. The deflection image is presented here to enhance this fact. Remarkably a kind of organization is observed at the upper layer which did not reveal a pattern. These PLSs formed are of sufficient rigidity to ensure the contact scan at high resolution without perturbation of the sheet with the tip.

Therefore, our AFM results suggest that POPC was the most suitable lipid matrix to reconstitute the Omp1. These PLSs need to be further investigate, particularly, the origin of the corrugations on its top. At this point we are not able to confirm if these structures are due to the arrangement of the protein in lattices or are originated from self aggregation of the protein. Nowadays, we are studying the optimal conditions to minimize the repulsion over the sheet in order to reach higher resolution images.

Acknowledgements

S.M. and O.D. are recipients of a fellowship of ‘‘Recerca i Docència de la U.B.’’. Financial support from the ‘‘Acció Gaspar de Portolà-02’’ from DURSI (Generalitat de Catalunya) is gratefully acknowledged. This work was supported by grants TIC202-04280-C03-01 and SAF2002-00698 (from DGICYT Spanish Ministry of Science and Technology/FEDER).

References

[1] J.L. Vázquez, S. Merino, Ò. Domènech, M. Berlanga, M. Viñas, M.T. Montero, J. Hernández-Borrell, Determination of the partition coefficient of a homologous series of ciprofloxacin: influence of the N-4 piperazinyl alkylation on the antimicrobial activity, Int. J. Pharm. 220 (2001) 53–62.

[2] W. Achouak, T. Heulin, J.M. Pagès, Multiple facets of bacterial porins, FEMS Microbiol. Lett. 199 (2001) 1–7.

[3] L. Sánchez, M. Puig, C. Fusté, J.G. Lorén, M. Viñas, Outer membrane permeability of non-typable *Haemophilus influenzae*, J. Antimicrob. Chemother. 37 (1996) 341–344.

[4] L. Sánchez, N. Ruiz, S. Leranoz, M. Viñas, M. Puig, The role of the outer membrane in *Serratia marcescens* intrinsic resistance to antibiotics, Microbiología vol. 13, SEM, Barcelona, Spain, 1997, pp. 315–320.

[5] M. Puig, C. Fusté, M. Viñas, Outer membrane proteins from *Serratia marcescens*, Can. J. Microbiol. 39 (1993) 108–111.

[6] M. Berlanga, N. Ruiz, J. Hernández-Borrell, M.T. Montero, M. Viñas, Role of the outer membrane in the accumulation of quinolones by *Serratia marcescens* Can, J. Microbiol. 46 (2000) 716–722.

[7] J.L. Vázquez, M.T. Montero, S. Merino, Ò. Domènech, M. Berlanga, M. Viñas, J. Hernández-Borrell, Location and nature of the surface membrane binding site of the antibiotic ciprofloxacin: a fluorescence study, Langmuir 17 (2001) 1009–1014.

[8] N. Ruiz, M.T. Montero, J. Hernández-Borrell, M. Viñas, N. Ruiz, The role of *Serratia marcescens* porins in antibiotic resistance, Microb. Drug Resist. 9 (2003) 257–264.

[9] N. Ruiz, E. Maier, C. Andersen, R. Benz, M. Viñas, Molecular and functional characterization of the *Serratia marcescens* outer membrane protein Omp1, Biophys. Chem. (2004) 215–227.

[10] W. Kühlbrandt, in: H. Schägger, C. Hunter (Eds.), Membrane protein purification and crystallization: a practical approach, Academic Press, San Diego, 2002, pp. 253–283.

[11] E.M. Landau, J.P. Rosenbusch, Lipidic cubic phases: a novel concept for the crystallization of membrane proteins, Proc. Natl. Acad. Sci. U. S. A. 93 (1996) 14532–14535.

[12] A.G. Lee, Lipid–protein interactions in biological membranes: a structural perspective, Biochim. Biophys. Acta 1612 (2003) 1–40.

[13] M. Regué, C. Fabregat, M. Viñas, Isolation and partial characterization of a generalized transducing bacteriophage for *Serratia marcescens*, Res. Microbiol. 142 (1991) 23–27.

[14] S.T. Cole, I. Sonntag, U. Henning, Cloning and expression in *Escherichia coli* k-12 of the genes for major outer membrane protein OmpA from *Shigella dysenteriae*, *Enterobacter aerogenes* and *Serratia marcescens*, J. Bacteriol. 149 (1982) 145–150.

[15] M.T. Montero, J. Hernández-Borrell, K. Nag, K.M.W. Keough, Fluoroquinolone distribution in a phospholipid environment studied by fluorimetry, Anal. Chim. Acta 290 (1994) 58–64.

[16] S. Merino, Ò. Domènech, I. Dìez, F. Sanz, M. Viñas, M.T. Montero, J. Hernández-Borrell, Effects of ciprofloxacin on *Escherichia coli* lipid bilayers: an atomic force microscopy study, Langmuir 19 (2003) 6922–6927.

[17] S. Merino, J.L. Vázquez, Ò. Doménech, M. Berlanga, M. Viñas, M.T. Montero, J. Hernández-Borrell, Fluorquinolone-biomembrane interaction at the DPPC/PG lipid-bilayer interface, Langmuir 18 (2002) 3288–3292.

[18] S. Scheuring, P. Ringler, M. Borgnia, H. Stahlberg, D.J. Müller, P. Engel, A. Engel, High resolution AFM topographs of the *Escherichia coli* water chanel aquaporin Z, EMBO J. 18 (1999) 4981–4987.

[19] A. Stamouli, S. Kafi, D.C.G. Klein, T.H. Oosterkamp, J.W.M. Frenken, R.J. Cogdell, T.J. Aartsma, The ring structure and organization of light harvesting 2 complexes in a reconstituted lipid bilayer, resolved by atomic force microscopy, Biophys. J. 84 (2003) 2483–2491.

[20] F. Kienberger, C. Stroh, G. Kada, R. Moser, W. Baumgartner, V. Pastusheko, C. Rankl, R. Schimdt, H. Müller, E. Orlova, C. LeGrimellec, D. Drenckhahn, D. Blaas, P. Hinterdorfer, Ultramicorscopy 97 (2003) 229–237.

[21] M. Behlam, D.J. Mills, H. Quader, W. Kühlbrandt, J. Vonck, Projection structure of the monomeric porin OmpG at 6 Å resolution, J. Mol. Biol. 305 (2001) 71–77.

[22] H. Kim, Crystallization of OmpC osmoporin from *Escherichia coli* Acta Cryst, Acta Cryst., (1998) D541399–D541400.

[23] K. Zeth, V. Schnaible, M. Przybylski, W. Welte, K. Diederichs, H. Engelhardt, Crystallization and preliminary X-ray crystallographic studies of the native and chemically modified anion-selective porin from *Comamonas acidovorans*, Acta Cryst. D54 (1998) 650–653.

[24] D.J. Müller, H.J. Sass, S.A. Müller, G. Büldt, A. Engel, Surface structures of native bacteriorhodopsin depend on the molecular packing arrangement in the membrane, J. Mol. Biol. 285 (1999) 1903–1909.

[25] M. Yeager, In situ two-dimensional crystallization of a polytopic membrane protein: the cardiac gap junction chanel, Acta. Cryst. D50 (1994) 632–638.

[26] H. Maeda, An atomic force microscopy study for the assembly structures of tobacco mosaic virus and their size evaluation, Langmuir 13 (1997) 4150–4161.
[27] R. García, M. Calleja, H. Rohrer, Patterning of silicon surfaces with noncontact atomic force microscopy: field-induced formation of nanometer-size water bridges, J. Appl. Phys. 86 (1999) 1898–1903.
[28] L. Rigaud, G. Mosser, J.J. Lacapere, A. Olofsen, D. Levy, J.L. Roneck, Bio-beads: an efficient strategy for two-dimensional crystallization of membrane proteins, J. Struct. Biol. 118 (1997) 226–235.