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Reconstitution and alignment by a magnetic field of a β-barrel membrane protein in bicelles

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Abstract A protocol is described for the reconstitution of a transmembrane β-barrel protein domain, tOmpA, into lipid bicelles. tOmpA is the largest protein to be reconstituted in bicelles to date. Its insertion does not prevent bicelles from orienting with their plane either parallel or perpendicular to the magnetic field, depending on the absence or presence of paramagnetic ions. In the latter case, tOmpA is shown to align with the axis of the β-barrel parallel to the magnetic field, i.e. perpendicular to the plane of the bilayer, an orientation conforming to that in natural membranes and favourable to structural studies by solid-state NMR. Reconstitution into bicelles may offer an interesting approach for structural studies of membrane proteins in a medium resembling a biological membrane, using either NMR or other biophysical techniques. Our data suggest that alignment in the magnetic field of membrane proteins included into bicelles may be facilitated if the protein is folded as a β-barrel structure.

Keywords Solid-state NMR · Membrane protein · Bicelle · Outer membrane protein A (OmpA) · Structure · Orientation

Abbreviations C_8E_4: *n*-Octyltetraoxyethylene · C_8POE: *n*-Octylpolyoxyethylene · CD: Circular dichroism · DoMPC: 1,2-Di-*O*-tetradecyl-*sn*-glycero-3-phosphocholine · DoHPC: 1,2-Di-*O*-hexyl-*sn*-glycero-3-phosphocholine · DHPC: 1,2-Di-hexyl- *sn*-glycero-3-phosphocholine · DMPE-DTPA: 1,2-Dimyristoyl-*sn*-glycero-3-phospho ethanolamine-*N*-DTPA · DMPS: 1,2-Dimyristoyl-*sn*-glycero-3-phosphoserine · MAS: Magic-angle spinning · MES: 2-morpholinoethanesulfonic acid · PISA: Polarity index slant angle · tOmpA: Transmembrane domain of Outer Membrane Protein A from *Escherichia coli* · Tris–HCl: 2-amino-2-hydroxymethyl-1,3-propanediol hydrochloride

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Introduction

Protein structure determination by solution-state NMR is applicable to membrane proteins in detergents or amphipols (for recent examples, see Howell et al. 2005; Roosild et al. 2005; Zoonens et al. 2005). In solution, however, the protein experiences an environment extremely different from the native one, and all or most of the information about its orientation relative to the membrane plane and its interactions with lipids is lost. Solid-state NMR is an alternative technique that is applicable to proteins embedded into lipid bilayers. Outstanding progress has recently been achieved in studying solid samples by magic-angle spinning (MAS) NMR (for recent examples, see Ritter et al. 2005; van Gammeren et al. 2005). Nevertheless, complete high-resolution structure determination of ^{13}C,^{15}N-labelled proteins by solid-state NMR remains, for the moment, a task that very few laboratories are able to achieve. Besides the multi-dimensional high-resolution NMR approaches, simpler experiments that would provide the orientation of proteins or protein segments relative to the membrane plane are still desirable. This can be achieved using mechanically oriented lipid bilayers on glass plates, ^{15}N-labelled membrane proteins and 2D-NMR experiments (Ketchem et al. 1993; Zeri et al. 2003; Kamihira et al. 2005). Lipid bicelles, however, may provide an attractive alternative.

Bicelles were proposed more than 10 years ago as a novel, promising membrane-mimicking environment, intermediate between bilayers and micelles (Sanders and Schwonek 1992). They occur when mixtures of short-

chain and long-chain lipids organise themselves into a discoid structure, with long-chain lipids forming a central bilayer and short-chain lipids forming the rim of the disk. Their ability to align in the magnetic field of an NMR spectrometer appeared as a potential asset for membrane protein structural studies. Several factors make bicelles conceivably more advantageous than glass plates for the study of aligned membranes: the sample volume occupied by the glass plates is replaced by water, the preparation is much easier, the sample is better hydrated and less subject to dehydration upon heating, the buffer composition can be changed at will, the samples are transparent and amenable to optical spectroscopy, etc. Nevertheless, after several promising early attempts, the number of successful protein structural studies in bicelles has remained disappointingly low. Several reasons can explain such a failure: first, most membrane proteins are fragile: their production, purification and reconstitution are delicate processes which often result in a sample loss; second, an understanding of the factors that control bicelle formation, alignment and stability over time as a function of composition, concentration and temperature has taken long to emerge (see Triba et al. 2005, and references therein); and third, the physico-chemical properties of bicelles and, in particular, their ability to align in a magnetic field, are modified by the presence of a peptide or protein in a way that is difficult to predict. For this reason, as well as for the sake of simplifying the biochemical task and limiting the complexity of the NMR spectra, previous attempts have focused on minimal proteins, namely small domains comprised of one or two transmembrane α-helices (Sanders and Landis 1995; Prosser et al. 1998; Glover et al. 2001; Whiles et al. 2002; De Angelis et al. 2004; Sizun et al. 2004).

We present here a successful attempt at reconstituting a larger membrane protein, the uniformly ^{15}N-labelled transmembrane domain of outer membrane protein A from *Escherichia coli* (tOmpA, 180 amino acids, 20 kDa), into lipid bicelles. tOmpA is one of the largest membrane proteins whose structure has been determined by solution NMR to date (Arora et al. 2001; Fernandez et al. 2001b; Zoonens et al. 2005). The present study makes it the largest to be reconstituted into bicelles. The protocol presented here has benefited from recent progress regarding the preparation of bicelles, the incorporation of lanthanide ions to flip their orientation (Prosser et al. 1996), the availability of lanthanide-chelating lipids that limit the diffusion of lanthanide ions on the surface of the bilayer (Prosser et al. 1998), the use of ether lipids to improve chemical stability (Ottiger and Bax 1999) and the addition of a small fraction of negatively charged phospholipids to create an electrostatic repulsion between bicelles (Struppe et al. 2000). Particular attention was given to develop a reconstitution procedure that would not lead to the denaturation or aggregation of membrane proteins. Our data show that incorporation of tOmpA does not prevent bicelles from orienting either parallel or perpendicular to the NMR magnetic field, depending on the absence or presence of paramagnetic ions. In the latter case, tOmpA orients, as expected, with the axis of the β-barrel parallel to the field. Although our results are quite preliminary in terms of resolution, these first ^{15}N NMR spectra indicate that the sensitivity is sufficient to study the orientation of the protein relative to the membrane, an information that is sometimes difficult to obtain otherwise.

Materials and methods

Materials

1,2-Di-*O*-tetradecyl-*sn*-glycero-3-phosphocholine (DoMPC), 1,2-di-*O*-hexyl-*sn*-glycero-3-phosphocholine (DoHPC), 1,2-dimyristoyl-*sn*-glycero-3-phosphoserine (DMPS) and the lanthanide chelating lipid 1,2-dimyristoyl-*sn*-glycero-3-phosphoethanolamine-*N*-DTPA (DMPE-DTPA) were purchased from Avanti Polar Lipids (Alabaster, AL, USA). Imidazole, Thulium (III) chloride hexahydrate, 2-amino-2-hydroxymethyl-1,3-propanediol hydrochloride (Tris–HCl) and 2-morpholinoethanesulfonic acid (MES) were purchased from Sigma (Saint Quentin Fallavier, France). *n*-octyltetraoxyethylene (C_8E_4) and *n*-octylpolyoxyethylene (C_8POE) were purchased from Bachem (Weil am Rhein, Germany).

Sample preparation

The ^{15}N-tOmpA, with an eight-residue polyhistidine tag at the C-terminus end, was expressed, refolded and purified as described previously (Pautsch et al. 1999; Zoonens et al. 2005). As final steps, C_8POE was first exchanged either for C_8E_4 (for circular dichroism experiments) or for DoHPC (for bicelle preparations), with tOmpA trapped on a 1ml HiTrap chelating column (Amersham, Orsay, France) through interaction between the polyhistidine tag and nickel ions from the column. tOmpA was then detached from the column using imidazole which was filtered out using a 5ml HiTrap desalting column (Amersham, Orsay, France). At this step, the pH and detergent concentration were adjusted. For circular dichroism samples, the pH was kept at 8 with a 20-mM Tris–HCl buffer and the concentration of C_8E_4 was 20 mM. For NMR samples, the pH was lowered to 6.5 with a 10-mM MES buffer and the concentration of DoHPC was 25 mM. The solution of DoHPC and protein was then added to a dry powder mixture of DoMPC, DMPS and DMPE-DTPA to reach a molar ratio q of 3 between the long-chain lipids (DoMPC, DMPS and DMPE-DTPA) and the short-chain one (DoHPC). The final molar ratio DoMPC/DMPS/DMPE-DTPA/DoHPC was 96/2/2/33. After four cycles of vortexing, freezing and thawing, a clear bicelle solution was obtained. At this stage, the lipid concentration was approximately 10% (w/w), as

deduced from measuring the sample volume for a known volume occupied by the lipids. To obtain a sample with 60% lipid concentration (w/w), water was evaporated using an argon gas stream and gently heating (35°C), while vortexing the sample to maintain its homogeneity. This method was preferred to rotary evaporation, which induces foaming and loss of part of the sample. During concentration, the sample integrity and q ratio are controlled, and adjusted if necessary, by ^{31}P NMR in a well-oriented phase domain. The final lipid concentration is 60% (w/w) and the final sample volume 320 µl, containing 190 mg of lipids and 1.2 mg of tOmpA.

Circular dichroism (CD)

Far UV CD spectra were recorded on a Jobin-Yvon CD6 dichrograph (Longjumeau, France). Four scans were accumulated for each spectrum from 200 to 250 nm, with an integration time of 1 s and a 0.2 nm step. Background spectra without tOmpA were subtracted and spectra normalised with respect to protein concentration.

NMR spectroscopy

^{15}N and ^{31}P spectra were acquired using a Bruker AVANCE DMX 400 MHz wide-bore NMR spectrometer (Wissembourg, France). ^{1} H-decoupled ^{31}P-NMR spectra were acquired at 162 MHz, either with a 10-mm Bruker broadband probe or with a 4-mm Bruker MAS probehead. For the 10-mm probehead, the insert that contained the sample was inserted in a classical NMR tube containing D_2O and a phosphate buffer for external reference. Typical acquisition parameters were as follows: ^{31}P (90) pulse lengths were 24 and 6 µs for the 10 and 4-mm probe, respectively, and decoupling was performed using WALTZ-16 with a very low ^{1} H decoupling power (10 kHz). Acquisition and repetition times were 200 ms and 5 s, respectively. Spectra were processed with an exponential line broadening of 10 Hz. Other details for improved sample alignment have been described elsewhere (Triba et al. 2005). Static cross-polarised ^{15}N spectra were acquired at 41 MHz with a 7-mm Bruker MAS probehead. The 7-mm MAS rotor, containing an insert, was mechanically blocked in the NMR probe to impede its rotation during acquisition. The ^{1} H (90) pulse length was 4 µs and the contact time was 1.5 ms. Continuous decoupling was performed at high power (30 kHz). Acquisition and repetition times were 26 ms and 3.5 s, respectively. Spectra were processed with an exponential line broadening of 150 Hz.

Simulations

Simulated ^{15}N chemical shift tensor parameters σ_{11}, σ_{22}, σ_{33} are respectively 64, 77 and 217 ppm (Wu et al. 1995). This corresponds to an isotropic chemical shift value (δ_{iso}) of 119 ppm, an anisotropic parameter value (δ_{aniso}) of 98 ppm and an asymmetric parameter value (η) of 0.13. Using the molecular frame conventions of reference (Vosegaard and Nielsen 2002), the σ_{22}-axis of this tensor coincides with the z-axis of the molecular frame while the angle between the σ_{33}-axis and the x-axis is 17°.

For a given protein orientation, the magnetic field orientation (θ,ϕ) in the principal axis system of the ^{15}N chemical shift tensor was determined for each residue of tOmpA, using the appropriate file from the Protein Data Bank. We have used the file 1G90 deduced from solution-state NMR experiments (Arora et al. 2001). The ^{15}N NMR spectra of tOmpA were then simulated by solving the classical Eq. 1 relating the resonance frequency (δ) and the angles (θ,ϕ). A line width of 5 ppm was taken for all ^{15}N lorentzian lines. When this main axis is parallel to the magnetic field, rotating the protein around this axis is irrelevant for ^{15}N NMR resonance frequency calculation. In other cases, ^{15}N NMR simulated spectra were obtained by integrating the previous expression over all possible orientations, with 1° step, around this axis.

$$\delta = \delta_{iso} + \delta_{aniso}.(3.\cos^2\theta - 1 - \eta.\sin^2\theta.\cos\phi)/2 \qquad (1)$$

Results and discussion

tOmpA-containing bicelles adopt a negative orientation in the magnetic field

Under our experimental conditions, the average bicelle is expected to comprise approximately 1,700 long-chain lipids and 600 short-chain ones, and to incorporate, on an average, one molecule of tOmpA, occupying less than 1% of the bicelle surface. ^{31}P NMR spectra show that these mixed assemblies do align in the magnetic field of the NMR spectrometer (Fig. 1a). The high-field and low-field peaks can be assigned to DoMPC and DoHPC phosphorus nuclei, respectively. Their positions indicate that, at 37°C, the mixed bicelles orient, as protein-free bicelles do, with the plane of the bilayer parallel to the magnetic field. The intermediate resonance at 2 ppm is that of the phosphate buffer used as an external standard.

The mixture of four lipids used for the present study features three different headgroups and two different chain lengths and backbone linkages (ester and ether). The use of 2% negatively charged lipids aims at mimicking the properties of cell membranes as well as stabilising bicelles by inducing an electrostatic repulsion to avoid aggregation. Despite this compositional complexity, bicelles obviously retain their disk structure and still align in the magnetic field in a significant temperature range (30–47°C). Bicelles are described here as discoidal particles although other morphologies have been proposed (perforated lamellae, wormlike micelles,

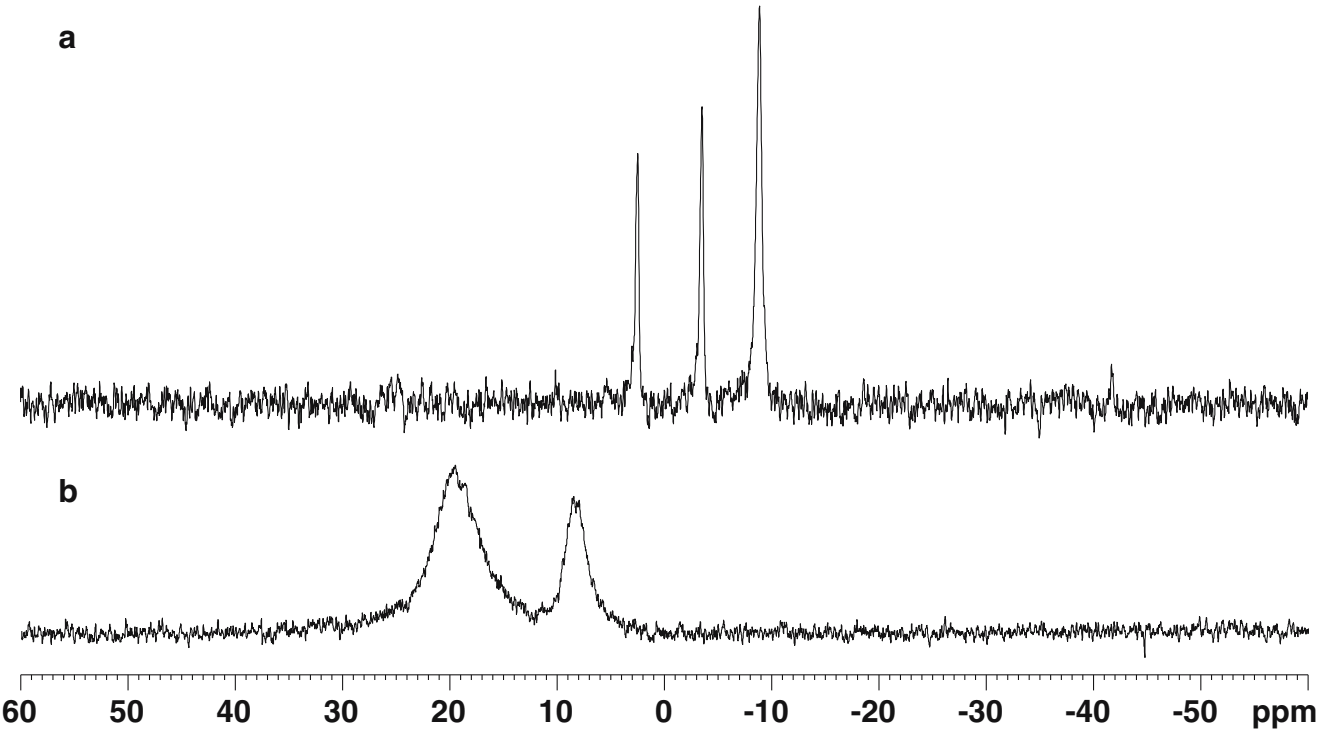

Fig. 1 ^{31}P NMR spectra of negatively (**a**) and positively (**b**) oriented bicelles containing ^{15}N-tOmpA. On spectrum (**a**) the resonance at 2 ppm corresponds to the external phosphate buffer standard [absent in (**b**)]. Spectrum (a) was acquired on a 10-mm solution NMR probe, on a diluted sample (200 µl; 35% lipids (w/w)) at 37°C. Spectrum (**b**) was acquired on a 4-mm solid-state NMR probe, on a concentrated sample [100 µl; 60% lipids (w/w)] at 40°C. Both spectra were obtained with approximately the same protein and lipid amount and were acquired with eight scans

ribbons etc.) as discussed before (Triba et al. 2005). In all cases, what is important here is that these particles contain a bilayer lamella domain that aligns in a magnetic field in a specific temperature range.

One advantage of bicellar phases is that they are optically clear, which makes them amenable to optical spectroscopy. The possibility that the protein denatures in the course of sample preparation can, therefore, be checked, even though relatively crudely, by comparing its secondary structure before and after reconstitution. Not surprisingly given the rugged character of tOmpA, its circular dichroism spectrum in bicelle mixtures is identical to that in C_8E_4 detergent solution, which is known to correspond to a folded, β-barrel protein (Fig. 2). At 10°C and 20% lipid concentration (w/w), bicelle mixtures do not form large disks that align in a magnetic field but small disks that tumble isotropically (Triba et al. 2005). Nevertheless, our point here is to show that tOmpA was not denatured despite changing the detergent, sample composition, dilution, buffer, pH and temperature.

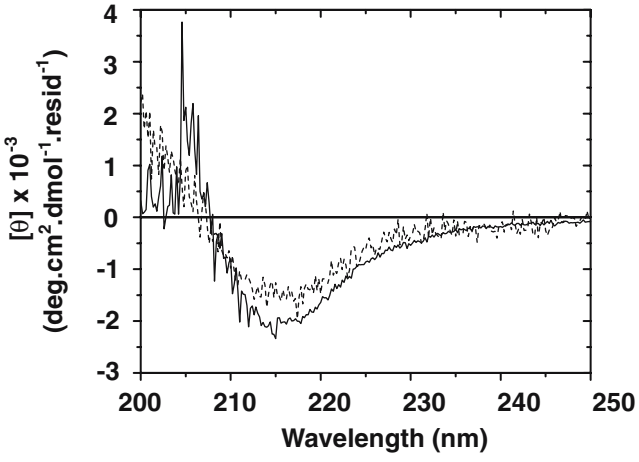

Fig. 2 Normalised circular dichroism spectra (mean residue molar ellipticity) of tOmpA at 10°C, before (*solid line*) and after (*dashes*) reconstitution in bicelles. *Solid line* 0.8 ml of 7.4 µM tOmpA, 20 mM C_8E_4 and 20 mM Tris–HCl pH 8 placed in a thermostated cuvette with a 0.5 cm pathlength. *Dashed line* 0.15 ml of 6 µM tOmpA, 19.2 mM DoMPC, 6.6 mM DoHPC, 0.4 mM DMPS, 0.4 mM DMPE-DTPA, 0.36 mM Tm^{3+} and 10 mM MES pH 6.5 placed in a thermostated cuvette with a 0.1 cm pathlength

tOmpA-containing bicelles with paramagnetic cations adopt a positive orientation in the magnetic field

The negative orientation of bicelles is not well suited to structural studies of transmembrane peptides or proteins. For one thing, if the rotation of the protein around an axis normal to the bicelle plane is not fast enough, NMR spectra will show slow exchange between different tensor orientations. Secondly, because of the orientation dependence of chemical shifts, the resolution is best in positively oriented bicelles, whose plane is normal to the

magnetic field. The latter orientation can be obtained by adding lanthanide ions, whose binding change the sign of the magnetic susceptibility anisotropy of the bicelles (Prosser et al. 1996).

Samples, therefore, were supplemented with thulium (Tm^{3+}) ions, at a concentration slightly lower (by 10%) than that of DMPE-DTPA. The final molar ratio of DoMPC/DoHPC/DMPS/DMPE-DTPA/tOmpA/Tm^{3+} was 96/33/2/2/0.03/1.8. A ^{31}P NMR spectrum was acquired to check that lanthanide ions indeed induce bicelles to flip (not shown). Samples were then concentrated until the lipid concentration was ~60% (w/w) and a 100 µl aliquot transferred to a 4-mm rotor. Figure 1b shows a ^{31}P NMR spectrum acquired at 40°C. Broadening of ^{31}P lines is expected in presence of lanthanide ions, even when adding chelating lipids (Prosser et al. 1998). At variance with data collected in the absence of Tm^{3+}, the most intense of the two peaks, assigned to DoMPC, has moved downfield from that assigned to DoHPC, showing that bicelles now orient with the plane of the bilayer perpendicular to the magnetic field.

Figure 3a shows the ^{15}N NMR spectrum of the same sample, acquired in a 7-mm rotor, at 35°C. While the signal-to-noise ratio and resolution are not optimal, the spectrum shape clearly suggests that the protein is oriented. The sensitivity, which is currently limited, could be improved for instance by resorting to improved polarisation transfer schemes (Kim et al. 2004). The ^{15}N resonance frequency of an amino acid depends on the orientation of the ^{15}N–^{1}H bond relative to the magnetic field. For an ideal and static α-helix with its axis parallel to the magnetic field, most amide ^{15}N resonate around 200 ppm. For an ideal and static β-barrel with its axis parallel to the magnetic field, ^{15}N resonance frequencies depend on the tilt angles of the β-strands. Because of the β-strand structure, ^{15}N–^{1}H bonds alternate between two orientations, resulting in two main resonance frequencies. By simulating ^{15}N spectra of a variety of β-barrel proteins, several authors have concluded that resonances are expected on a wide range of frequencies, usually with two maxima, one around 100 and the other around 200 ppm (Marassi 2001; Vosegaard and Nielsen 2002; Bleile et al. 2005). In addition, amino acids in mobile regions (loops, N- and C-termini) should contribute to resonances mainly between 50 and 130 ppm (Marassi and Opella 2003; Kamihira et al. 2005).

Our own simulations show that the resonance at 175 ppm is characteristic of tOmpA lying with the axis of the β-barrel oriented parallel to the magnetic field (Fig. 3b). If we simulate tOmpA slightly tilted away from the direction of the field, while the resonance at ca. 100 ppm is not affected, the peak at 175 ppm shifts to the right (not shown). If the tilt exceeds ~5°, the low field resonance merges with the high field resonance and the simulated spectrum resembles that of Fig. 3c, obtained by simulating tOmpA lying perpendicular to

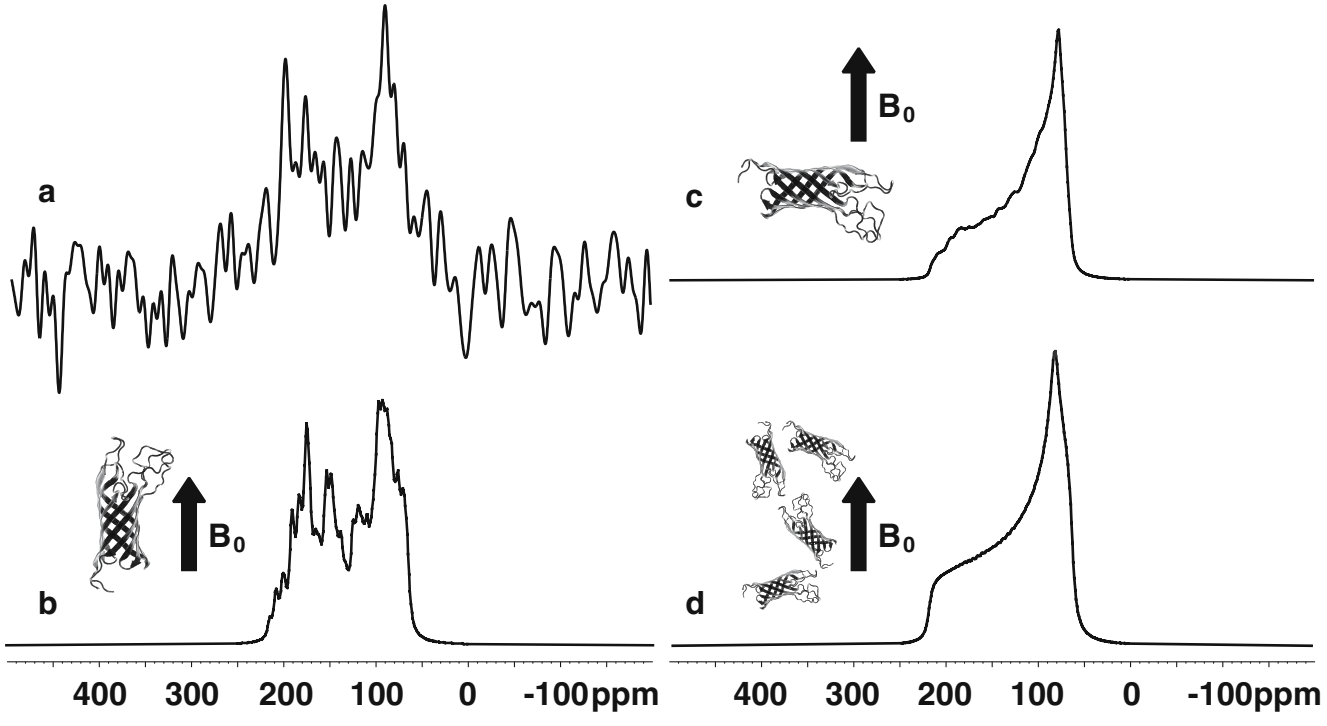

Fig. 3 **a** ^{15}N NMR spectrum of 1.2 mg ^{15}N-tOmpA inside positively oriented bicelles. The spectrum was acquired on a 7-mm solid-state NMR probe, on a concentrated sample [320 µl, 60% lipids (w/w)] at 35°C, with 40,000 scans (overall acquisition time: 40 h). **b** Numerical simulation of the same spectrum with tOmpA aligned with its main axis parallel to the magnetic field. **c** Numerical simulation of the same spectrum with tOmpA aligned with its main axis perpendicular to the magnetic field. **d** Numerical simulation of a powder spectrum (see cartoons, for details see text)

the magnetic field. Of course, bicelles themselves are distributed and oscillate in a cone around their average position, but this mostly affects the ^{15}N resonance line widths rather than their frequency. The singularity at ca. 175 ppm is also absent in a powder spectrum, obtained when all protein orientations are equally possible (Fig. 3d). These observations are consistent with the expected transmembrane orientation of tOmpA.

Resonances besides those at 100 and 175 ppm result either from amino acids in the loops or in regions of the barrel that are significantly distorted. For example, our simulated spectrum using tOmpA structural data from solution NMR (Fig. 3b) shows an additional peak at ca. 150 ppm that results from 19 amino acids, including eight odd-numbered amino acids which correspond to a flattening of the barrel on one side (not shown). Higher sensitivity would be required to deduce from our solid-state NMR spectrum that such a structural feature is present in our sample. The fact that an additional resonance next to the low field one is also visible in Fig. 3a indicates that it may be the case. In addition, such additional resonances offer potential help for spectral assignment of better quality 1D or 2D solid-state NMR spectra. One has to keep in mind, however, that simulations rely on a number of parameters, not all of which are unambiguously determined. For one thing, if the simulation relies on a structure determined by X-ray crystallography, proton positions must often be predicted from that of the other atoms. Furthermore, structural and dynamic differences are expected between the crystalline and the NMR samples, especially in the loops. In addition, chemical shift tensors slightly depend on amino acids and their environment, a fact that has been neglected so far although such variations may affect ^{15}N-NMR simulated spectra (Marassi 2001; Vosegaard and Nielsen 2002; Bleile et al. 2005). With these qualifications in mind, our data nevertheless indicate that the simulation of NMR data obtained on oriented bicelles may yield a relatively precise evaluation of a protein's orientation with respect to the membrane, an information that may be difficult to obtain otherwise (Rodionova et al. 1995).

Our ^{15}N NMR data unambiguously show that the protein orients, in the presence of Tm^{3+} ions, with the axis of the β-barrel parallel to the field. This is an indication that a substantial fraction of tOmpA (if not all of it) indeed is incorporated in the bicelles. Taken together, the distinct ^{31}P spectra recorded either in the absence or presence of Tm^{3+}, therefore, show that the presence of tOmpA does not prevent the bicelles from orienting in one or the other direction, while the ^{15}N spectrum shows that the orientation of the bicelles imposes that of the protein. The ^{15}N NMR spectrum also shows that the sensitivity of such an experiment is not prohibitively low for structural studies. As expected from simulations, the resolution of our 1D spectrum indicates that a detailed structural study would require either higher spectral dimensionality and/or selective ^{15}N-labelling schemes.

In the course of this work, we noted that the amount of lanthanide ions required to flip tOmpA-containing bicelles from their negative to their positive orientation is twice of that needed in the absence of tOmpA. This can possibly be explained by the presence of an eight-histidine tag at the C-terminus of the protein, which can also chelate lanthanide ions (final samples contain DMPE-DTPA, histidine residues and Tm^{3+} in a 2/0.24/1.8 molar ratio). Because of the fast and quasi-isotropic motion expected for tags, any Tm^{3+} ions they chelate would likely have little effect on bicelle orientation. In addition, the contribution of tOmpA may increase the magnetic susceptibility anisotropy of the bicelles, so that the adsorption of more lanthanide ions would be necessary for changing its sign.

Conclusion

The present data support the view that bicelles indeed do represent an interesting alternative to glass plates for membrane alignment in view of solid-state NMR measurements. While the possibility of achieving spectra of a quality sufficient for membrane protein structure determination remains to be demonstrated, studying the orientation of a protein relative to the membrane plane clearly is within the reach of such experiments. Our reconstitution protocol, applied here to the largest protein to be incorporated in bicelles, has been designed so as to be as mild as possible to membrane proteins, most of which tolerate freeze-thawing—likely to be the most traumatic step—as long as they are in a membrane-like environment. It may probably serve as a guideline for future attempts at incorporating membrane proteins into bicelles in view of NMR studies, as well as for investigations by other spectroscopic methods, where bicelles can be used as an optically transparent membrane-mimicking medium.

One risk that should not be overlooked is that, once a membrane protein has been reconstituted into bicelles, it migrates preferentially towards the rims, where it could either denature or misorient. This is known to happen for α-helical peptides with lytic properties, such as melittin. Such a distribution would also favour a shrinking of the bicelles, which could compromise their ability to orient. Our CD and ^{31}P- and ^{15}N-NMR data show that, in the case of tOmpA and under the present experimental conditions, none of these events takes place. It may be that bicelles are particularly appropriate for the study of β-barrel proteins: the fact that no studies have been published to date of whole α-helical membrane proteins in lipid bicelles may perhaps be taken as an indication that a successful incorporation of such proteins may be more difficult to achieve, or that the magnetic susceptibility anisotropy of transmembrane α-helices complicates bicelle alignment in a magnetic field (see Worcester 1978). Interestingly, recent studies have shown that 2D ^{15}N, ^{1}H-NMR of aligned β-barrels is a good approach to obtaining structural information

via the (polarity index slant angle PISA) experiment (which correlates the ^{15}N chemical shift spectrum with a ^{15}N–^{1}H dipolar coupling spectrum) (Marassi and Opella 2000; Marassi 2001; Vosegaard and Nielsen 2002; Bleile et al. 2005).

It is also worth noting that DHPC (1,2-di-hexyl- *sn*-glycero-3-phosphocholine) has recently become a detergent of choice for structural studies of membrane proteins by solution-state NMR (Fernandez et al. 2001a). In solution NMR studies, it is typical to use molar ratios of detergent to protein in the range of 100–300:1. The DoHPC/tOmpA molar ratio used in the present study is ~100:1. It would thus be straightforward, once solution data have been collected, to supplement the DHPC- or DoHPC-solubilised protein with long-chain lipids, which would yield samples permitting the formation of bicelles and alignment of the proteins in the magnetic field. This would open the way to complementing high-resolution structural data obtained in solution with information on protein orientation relative to the membrane, using the same protein sample and a combination of solution-state and solid-state NMR.

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References

Arora A, Abildgaard F, Bushweller JH, Tamm LK (2001) Structure of outer membrane protein A transmembrane domain by NMR spectroscopy. Nat Struct Biol 8:334–338

Bleile DW, Scott WR, Straus SK (2005) Can PISEMA experiments be used to extract structural parameters for mobile beta-barrels? J Biomol NMR 32:101–111

De Angelis AA, Nevzorov AA, Park SH, Howell SC, Mrse AA, Opella SJ (2004) High-resolution NMR spectroscopy of membrane proteins in aligned bicelles. J Am Chem Soc 126:15340–15341

Fernandez C, Adeishvili K, Wuthrich K (2001a) Transverse relaxation-optimized NMR spectroscopy with the outer membrane protein OmpX in dihexanoyl phosphatidylcholine micelles. Proc Natl Acad Sci USA 98:2358–2363

Fernandez C, Hilty C, Bonjour S, Adeishvili K, Pervushin K, Wüthrich K (2001b) Solution NMR studies of the integral membrane proteins OmpX and OmpA from Escherichia coli. FEBS Lett 504:173–178

van Gammeren AJ, Hulsbergen FB, Hollander JG, Groot HJ (2005) Residual backbone and side-chain 13C and 15N resonance assignments of the intrinsic transmembrane light-harvesting 2 protein complex by solid-state magic angle spinning NMR spectroscopy. J Biomol NMR 31:279–293

Glover KJ, Whiles JA, Wood MJ, Melacini G, Komives EA, Vold RR (2001) Conformational dimorphism and transmembrane orientation of prion protein residues 110–136 in bicelles. Biochemistry 40:13137–13142

Howell SC, Mesleh MF, Opella SJ (2005) NMR structure determination of a membrane protein with two transmembrane helices in micelles: MerF of the bacterial mercury detoxification system. Biochemistry 44:5196–5206

Kamihira M, Vosegaard T, Mason AJ, Straus SK, Nielsen NC, Watts A (2005) Structural and orientational constraints of bacteriorhodopsin in purple membranes determined by oriented-sample solid-state NMR spectroscopy. J Struct Biol 149:7–16

Ketchem RR, Hu W, Cross TA (1993) High-resolution conformation of gramicidin A in a lipid bilayer by solid-state NMR. Science 261:1457–1460

Kim H, Cross TA, Fu R (2004) Cross-polarization schemes for peptide samples oriented in hydrated phospholipid bilayers. J Magn Reson 168:147–152

Marassi FM (2001) A simple approach to membrane protein secondary structure and topology based on NMR spectroscopy. Biophys J 80:994–1003

Marassi FM, Opella SJ (2000) A solid-state NMR index of helical membrane protein structure and topology. J Magn Reson 144:150–155

Marassi FM, Opella SJ (2003) Simultaneous assignment and structure determination of a membrane protein from NMR orientational restraints. Protein Sci 12:403–411

Ottiger M, Bax A (1999) Bicelle-based liquid crystals for NMR-measurement of dipolar couplings at acidic and basic pH values. J Biomol NMR 13:187–191

Pautsch A, Vogt J, Model K, Siebold C, Schulz GE (1999) Strategy for membrane protein crystallization exemplified with OmpA and OmpX. Proteins 34:167–172

Prosser RS, Hunt SA, DiNatale JA, Vold RR (1996) Magnetically aligned membrane model systems with positive order parameter: Switching the sign of *Szz* with paramagnetic ions. J Am Chem Soc 118:269–270

Prosser RS, Volkov VB, Shiyanovskaya IV (1998) Novel chelate-induced magnetic alignment of biological membranes. Biophys J 75:2163–2169

Ritter C, Maddelein ML, Siemer AB, Luhrs T, Ernst M, Meier BH, Saupe SJ, Riek R (2005) Correlation of structural elements and infectivity of the HET-s prion. Nature 435:844–848

Rodionova NA, Tatulian SA, Surrey T, Jahnig F, Tamm LK (1995) Characterization of two membrane-bound forms of OmpA. Biochemistry 34:1921–1929

Roosild TP, Greenwald J, Vega M, Castronovo S, Riek R, Choe S (2005) NMR structure of Mistic, a membrane-integrating protein for membrane protein expression. Science 307:1317–1321

Sanders CR, 2nd, Schwonek JP (1992) Characterization of magnetically orientable bilayers in mixtures of dihexanoylphosphatidylcholine and dimyristoylphosphatidylcholine by solid-state NMR. Biochemistry 31:8898–8905

Sanders CR, 2nd, Landis GC (1995) Reconstitution of membrane proteins into lipid-rich bilayered mixed micelles for NMR studies. Biochemistry 34:4030–4040

Sizun C, Aussenac F, Grelard A, Dufourc EJ (2004) NMR methods for studying the structure and dynamics of oncogenic and antihistaminic peptides in biomembranes. Magn Reson Chem 42:180–186

Struppe J, Whiles JA, Vold RR (2000) Acidic phospholipid bicelles: a versatile model membrane system. Biophys J 78:281–289

Triba MN, Warschawski DE, Devaux PF (2005) Reinvestigation by phosphorus NMR of lipid distribution in bicelles. Biophys J 88:1887–1901

Vosegaard T, Nielsen NC (2002) Towards high-resolution solid-state NMR on large uniformly 15N- and [13C,15N]-labeled membrane proteins in oriented lipid bilayers. J Biomol NMR 22:225–247

Whiles JA, Glover KJ, Vold RR, Komives EA (2002) Methods for studying transmembrane peptides in bicelles: consequences of hydrophobic mismatch and peptide sequence. J Magn Reson 158:149–156

Worcester DL (1978) Structural origins of diamagnetic anisotropy in proteins. Proc Natl Acad Sci USA 75:5475–5477

Wu CH, Ramamoorthy A, Gierasch LM, Opella SJ (1995) Simultaneous characterization of the Amide 1H Chemical Shift, 1H-15N Dipolar, and 15N chemical shift interaction tensors in a peptide bond by Three-dimensional solid-state NMR Spectroscopy. J Am Chem Soc 117:6148–6149

Zeri AC, Mesleh MF, Nevzorov AA, Opella SJ (2003) Structure of the coat protein in fd filamentous bacteriophage particles determined by solid-state NMR spectroscopy. Proc Natl Acad Sci U S A 100:6458–6463

Zoonens M, Catoire LJ, Giusti F, Popot JL (2005) NMR study of a membrane protein in detergent-free aqueous solution. Proc Natl Acad Sci U S A 102:8893–8898