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Rotational dynamics of spin-labelled surfactant-associated proteins SP-B and SP-C in dipalmitoylphosphatidylcholine and dipalmitoylphosphatidylglycerol bilayers

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Abstract

Pulmonary surfactant proteins SP-B and SP-C have been isolated from porcine lungs and selectively labelled with 2,2,6,6-tetramethylpiperidine-*N*-oxyl (TEMPO)-isothiocyanate at their N-terminal amine ends, to analyse the mobility of both proteins on the nanosecond time scale using electron spin resonance (ESR) spectroscopy. Reconstitution of the labelled forms of these proteins in bilayers of dipalmitoylphosphatidylcholine (DPPC) or dipalmitoylphosphatidylglycerol (DPPG) results in much broader and anisotropic ESR spectra, indicating a large restriction in rotational mobility of the protein-attached probe when inserted in membranes. Distinctive differences were found between the ESR spectra of the two polypeptides, that were consistent with intrinsic differences in mode of interaction of SP-B and SP-C with phospholipid bilayers. The mobility of the protein spin probes was sensitive to temperature on the time scale of conventional spin-label ESR. Both proteins, TEMPO-SP-B and TEMPO-SP-C, showed considerable increases in mobility at temperatures above the pretransition of pure DPPC. Finally, the mobility of the spin probes attached to both SP-B and SP-C was more restricted in DPPG than in DPPC bilayers, demonstrating that electrostatic interactions of the positively charged residues at the protein surface influence the rotational dynamics of the proteins in anionic lipid bilayers. Although some residual segmental mobility of the thiourea-linked probes cannot be discounted, the results clearly reflect preferential differences in overall protein dynamics in gel and fluid phases of the two phospholipids that could be important for the biophysical properties of surfactant bilayers and monolayers.

Keywords: Pulmonary surfactant; Electron spin resonance; Labeled protein; Membrane dynamics; Lipid-protein interaction

1. Introduction

Pulmonary surfactant-associated proteins SP-B and SP-C are two hydrophobic peptides widely recognized as essential components for the biophysical properties of lung surfactant (for recent reviews on surfactant see [1–3]). This material has the important function of stabilizing the alveolar surface of lungs against the physical forces developed at the air-liquid interface throughout the respiratory cycle. In the ab-

Abbreviations: DPPC, 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine; DPPG, 1,2-dipalmitoyl-*sn*-glycero-3-phospho-*rac*-glycerol; EDTA, ethylenediaminotetraacetic acid; ESR, electron spin resonance; HEPES, *N*-[2-hydroxyethyl]piperazine-*N*′-[2-ethanesulphonic acid]; MALDI, matrix-assisted laser desorption ionization; PG, 1,2-diacyl-*sn*-glycero-3-phospho-*rac*-glycerol; SP-B, surfactant protein B; SP-C, surfactant protein C; TEMPO, 2,2,6,6-tetramethylpiperidine-*N*-oxyl; TEMPO-NCS, 4-isothiocyanato-TEMPO

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sence of tensoactive materials, the high surface tension of the thin water film lining the respiratory epithelium would lead to alveolar collapse at the lowest tidal volumes. Pulmonary surfactant dramatically reduces this interfacial tension by depositing a phospholipid-enriched monomolecular layer at the air-liquid interface. Although the main tensoactive component of surfactant is a disaturated species of phosphatidylcholine, dipalmitoylphosphatidylcholine (DPPC), presence of the hydrophobic peptides SP-B and SP-C is essential to promote rapid formation of the surfactant monolayer and to sustain the highest surface pressures (i.e. lowest surface tensions) of the interface that are required to keep the respiratory surface operational [4–6].

Numerous studies have approached the characterization of lipid-protein interactions with these hydrophobic proteins reconstituted in surfactant phospholipid bilayers [7–14] and monolayers [15–22]. This has been done in an attempt to identify the precise effects that these proteins have on the surfactant lipid components and that are associated with the tensoactive function. The understanding of such chemicophysical phenomena in molecular terms will allow the future design of new surfactant materials to be used in therapeutic treatments of surfactant-defective pathologies [23,24].

Previously we have studied the interaction of spin-labelled phospholipids with SP-B and SP-C in bilayers of DPPC and of the other phospholipid of major relevance in surfactant, viz. dipalmitoylphosphatidylglycerol (DPPG) [6,10]. The present work focusses on a study of the dynamic behaviour of the hydrophobic proteins SP-B and SP-C themselves in surfactant phospholipid bilayers. This has been realised by covalent attachment of spin probes to the proteins and analysis of the effects of protein insertion in phospholipid bilayers of DPPC and of DPPG on the electron spin resonance (ESR) spectra of the spin-labelled proteins.

2. Experimental

2.1. Materials

Chloroform and methanol were high performance liquid chromatography (HPLC) grade solvents from Scharlau (Barcelona, Spain). Sephadex LH-20 and LH-60 chromatography gels were obtained from Pharmacia (Uppsala, Sweden). DPPC and DPPG were from Avanti Polar Lipids (Birmingham, AL, USA). Spin-labelled phospholipids with the nitroxide group at positions 5 and 12 of the *sn*-2 acyl chain were synthesized as described by Marsh and Watts [25]. 4-Isothiocyanato-(2,2,6,6-tetramethylpiperidine-*N*-oxyl) (4-isothiocyanato-TEMPO) was from Sigma (St. Louis, MO, USA).

2.2. Isolation of SP-B and SP-C

Surfactant proteins SP-B and SP-C were isolated from porcine lungs by an adaptation of the method of Curstedt et al. [26], described elsewhere [27]. The purity of protein fractions was routinely checked by electrophoresis in SDS-polyacrylamide gels (4% and 18% acrylamide in stacking and separating gels, respectively). Quantitation of isolated proteins was carried out by amino acid analysis. Purified SP-B and SP-C fractions showed similar biophysical activity in promoting phospholipid interfacial adsorption as that previously described [5,6,28].

2.3. Protein spin-labelling

Covalent attachment of a 2,2,6,6-tetramethylpiperidine-*N*-oxyl (TEMPO) spin probe to SP-B and SP-C was achieved by reaction of the isothiocyanate derivative with the protein, in organic solution, at weakly alkaline pH, as previously described [21,22]. Briefly, a stock solution containing around 1 mg SP-B or SP-C in LH-60 elution solvent (chloroform/methanol/HCl 0.1 N, 1:1:0.05 v/v/v) was adjusted to apparent pH 7.5–7.8 with methanolic 50 mM Tris-HCl. Then 400 μg of 4-isothiocyanate-TEMPO was added and the mixture was incubated at 20°C overnight. Afterwards, the incubation solution was concentrated to a final volume of 0.5 ml and applied to a LH-20 column to separate the labelled protein from non-bound spin label reagent. Quantitation of protein-attached spin groups estimated that around 0.6–0.9 moles of TEMPO were incorporated per mole of SP-B or SP-C. Previous optimization of the labelling reaction conditions confirmed that precise control of pH to values below 8.0 during derivatization is critical (i) to preferentially label the N-terminal amine groups of

the proteins, and (ii) to conserve the palmitic chains esterifying the SP-C cysteines. MALDI-mass spectrometry analysis of protein samples modified by this procedure confirmed that the resulting preparation consisted of a mixture of non-labelled and single probe-labelled protein, the main form of SP-C being acylated with two palmitic chains. N-terminal sequencing of the modified proteins by Edman degradation produced only limited yields, suggesting partial blocking of their N-terminal amine as a consequence of the derivatization. Secondary structure of the modified proteins as analysed by circular dichroism was indistinguishable from that of the native forms. N-Terminal isothiocyanate-derivatized proteins were found to retain their surface active properties, to promote interfacial phospholipid adsorption and to modulate monolayer behaviour under dynamic compression, as previously reported [21,22].

2.4. Reconstitution of lipid/protein samples

After mixing appropriate amounts of spin-labelled proteins and phospholipid, DPPC or DPPG, in chloroform/methanol (2:1, v/v) solution, the solvent was evaporated under N_2 and the samples were dried under vacuum in a desiccator overnight. Samples were then hydrated in 100 μl of 50 mM HEPES buffer pH 7, containing 150 mM NaCl and 5 mM EDTA, at 50°C for 1 h, with occasional vortexing. The reconstituted material was then pelleted by centrifugation at 5000 rpm, in a bench centrifuge, into 100 μl capillary tubes for ESR spectroscopy, the pellet being washed with buffer and resuspended three times to remove possible traces of free probe. After the ESR experiments, the samples were again suspended in the same buffer and aliquots were taken to quantify protein and lipid. All samples in these experiments were reconstituted at relatively low protein-to-lipid ratio, 8% of labelled protein with respect to DPPC or DPPG (w/w), in order to minimize possible protein self-aggregation in the membranes. Under these conditions, the totality of the protein was associated with the membranes as detected by coflotation of lipid and protein after centrifugation of lipid/protein samples in ^2H_2O buffer [13].

2.5. ESR spectroscopy

ESR spectra were recorded as previously described [10,14], on a Varian E-12 Century Line 9 GHz spectrometer equipped with nitrogen gas flow temperature regulation. The capillaries were placed in a quartz tube containing light silicone oil for thermal stability. Temperature was measured with a fine-wire thermocouple positioned in the silicone oil at the top of the microwave cavity. Spectra were digitized on an IBM PC computer with Labmaster interface. Instrumental settings were 10 mW microwave power, 2 G modulation amplitude, 100 kHz modulation frequency, 0.25 s time constant, 4 min scan time, 100 G scan range, and 3245 G centre field. Several scans, typically three to five, were accumulated to improve signal-to-noise ratio.

3. Results and discussion

3.1. Spin-labelled proteins in organic solution

Fig. 1 presents the conventional ESR spectra of TEMPO-labelled SP-B and SP-C as organic solutions in chloroform/methanol (2:1, v/v), at 20°C. The sharp lines of the spectra indicate rapid, nearly isotropic motion of the spin label attached to both proteins, in this environment. An isotropic rotational correlation time (T_R) can be calculated from these spectra according to [29]:

$$T_R(s) = 6.5\times10^{-10}\Delta H_0[(h_0/h_{-1})^{1/2}-1] \quad (1)$$

where ΔH_0 is the peak-to-peak width of the central line, and h_0 and h_{-1} are the amplitude of the central field and high field lines, respectively (see Fig. 1). This dynamic parameter is sensitive to motion in the region probed by the spin label. The correlation times calculated this way were 0.04 and 0.11 ns for TEMPO-SP-B and TEMPO-SP-C respectively, close to the correlation time of free TEMPO-NCS probe in the same solvent, viz. 0.02 ns. These values are of the same order as those found by other authors for spin-labelled small peptides freely tumbling in different environments [30–34]. For comparison, the rotational correlation times for the whole protein in chloroform/methanol, calculated from the molecular

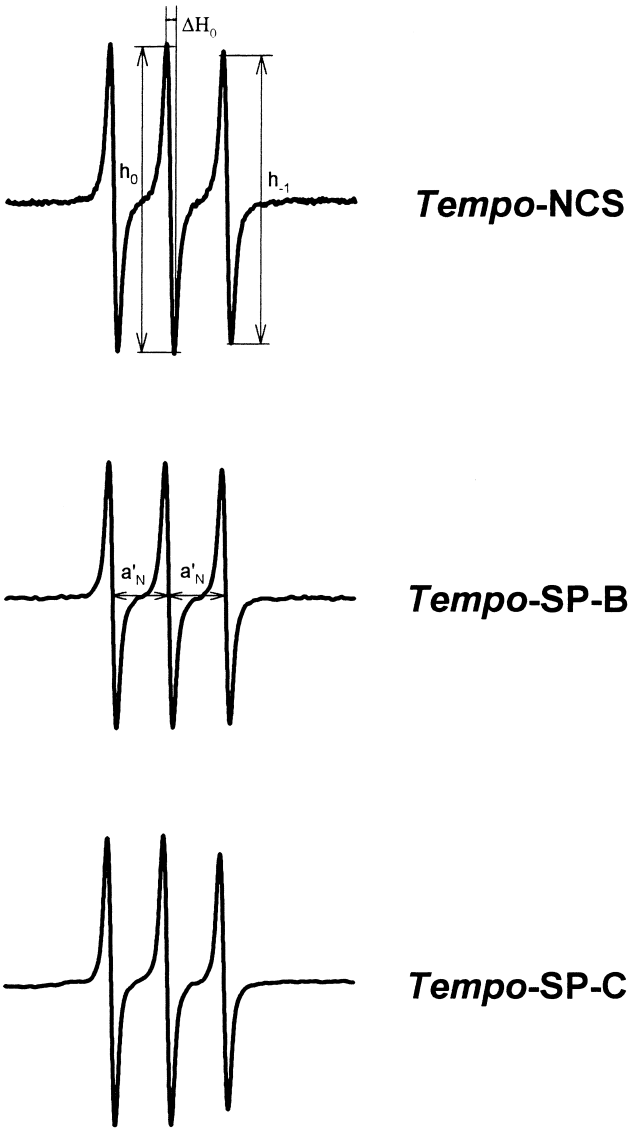

Fig. 1. Conventional ESR spectra of TEMPO-isothiocyanate and TEMPO-labelled SP-B and SP-C in chloroform/methanol 2:1 (v/v). Spectral parameters h_0, h_{-1} and ΔH_0 and a'_N were determined from the spectra as indicated. $T = 20°C$; total scan width, 100 G.

weights and the Debye equation for a compact quasi-spherical structure, are 3.1 and 0.75 ns for the SP-B dimer and SP-C monomer, respectively. The isotropic hyperfine coupling constant, a'_N, a parameter which is sensitive to the polarity of the environment, can be determined from the baseline crossings of the ESR spectra as shown in Fig. 1. The a'_N parameter for TEMPO-SP-B and TEMPO-SP-C in chloroform/methanol is 16.4 and 16.3 G respectively, also similar to the coupling constant for free TEMPO-NCS in chloroform/methanol (2:1, v/v), 16.2 G. The similarity of these values suggests that, although SP-B and SP-C have a defined secondary structure in these solvents [35,36], the labelled proteins expose their spin label groups to a solvent environment similar to that of the free probe. The short rotational correlation times of both TEMPO-labelled proteins and the fact that the shortest T_R belongs to TEMPO-SP-B, a protein which in its dimeric form is more than four times larger than TEMPO-SP-C, suggest that a substantial part of the mobility of the attached probe is segmental in origin. This is consistent with the exposed location of the probe that was inferred from the isotropic hyperfine splittings.

3.2. Spin-labelled proteins in phospholipid bilayers

Fig. 2 presents the ESR spectra of TEMPO-SP-B and TEMPO-SP-C reconstituted in bilayers of DPPC at temperatures below (20°C) and above (45°C) the temperature of gel-to-liquid crystalline phase transition of the lipid (41°C). The spectra are compared in the figure with the ESR spectrum of free TEMPO-NCS in the same reconstitution buffer, 50 mM HEPES containing 150 mM NaCl and 5 mM EDTA pH 7.0. The diagnostic features of the ESR spectral line shapes for both spin-labelled proteins in

Table 1
ESR spectral splittings of TEMPO-NCS and TEMPO-labelled SP-B and SP-C in organic solution, and reconstituted in bilayers of DPPC or DPPG, at temperatures below and above the phase transition of the phospholipid

	Environment	T (°C)	$2A_{max}$ (G)
TEMPO-NCS	Chl/MeOH	20	32.4[a]
TEMPO-SP-B	Chl/MeOH	20	32.7[a]
TEMPO-SP-C	Chl/MeOH	20	32.5[a]
TEMPO-NCS	HEPES buffer	20	33.6[a]
TEMPO-NCS	HEPES buffer	45	33.0[a]
TEMPO-SP-B	DPPC	20	59.7
TEMPO-SP-B	DPPC	45	47.4
TEMPO-SP-B	DPPG	20	63.2
TEMPO-SP-B	DPPG	45	55.1
TEMPO-SP-C	DPPC	20	59.7
TEMPO-SP-C	DPPC	45	43.5
TEMPO-SP-C	DPPG	20	64.0
TEMPO-SP-C	DPPG	45	52.8

[a]Isotropic hyperfine splitting, $2a'_N$.

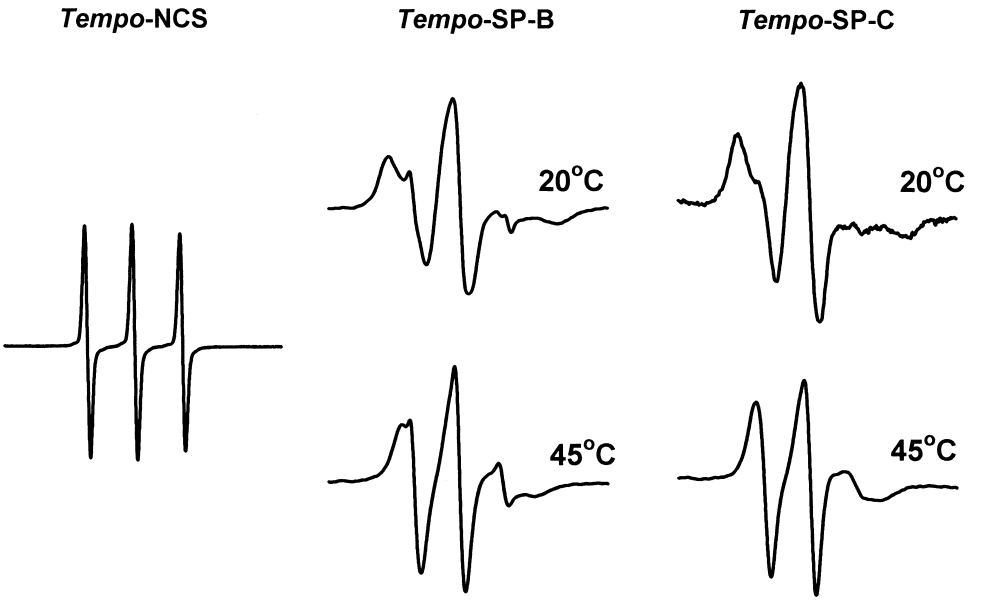

Fig. 2. ESR spectra of free TEMPO-isothiocyanate in 50 mM HEPES, 150 mM NaCl pH 7 and TEMPO-labelled SP-B and SP-C reconstituted in DPPC bilayers at temperatures below (20°C) or above (45°C) the gel-to-liquid crystalline phase transition temperature (41°C) of the phospholipid. Protein-to-lipid ratio was 8% (w/w) in all the samples. Total scan width of each spectrum, 100 G.

DPPC are indicative of a large restriction in the mobility of the probe in this environment. Similar spectral features have been discussed in other studies as indicative of penetration of a spin-labelled peptide into phospholipid bilayers [34]. Both TEMPO-SP-B and TEMPO-SP-C spectra show a marked anisotropy in DPPC bilayers, with well-defined outer hyperfine splittings (see Table 1). The spectral line shapes at 45°C are characteristic of preferential rotation of the probes about a particular axis. The ESR spectra of TEMPO-SP-B in DPPC bilayers contain a minor component with sharper lines, somewhat similar to that of the free probe. Spectral subtraction shows that this minor component consists of less than 1% of the total intensity and is attributed to free probe or a very small degree of derivatization of the ε-amino groups of lysine. Although the solubility of SP-B in aqueous solution is very limited [27], possible traces of free protein cannot be excluded. This minor component is absent from the spectra of SP-C, which contains fewer lysine residues.

The large restriction in mobility of the spin probe upon reconstitution of the labelled proteins in phospholipid bilayers can be due either to immobilization of the protein once inserted in the bilayer, or to immobilization of the probe itself, if embedded in the lipid matrix. The ESR spectra of both TEMPO-SP-B and TEMPO-SP-C are very similar at 25°C, a temperature in the gel phase of DPPC, suggesting that in both cases the slow motion of the probe could be mainly due to severe restrictions imposed by the tight packing of phospholipid molecules in the DPPC gel phase. On the other hand, the spectra of both spin-labelled SP-B and SP-C have considerably smaller hyperfine splittings at temperatures above the gel-to-fluid phase transition of the host phospholipid, viz. 45°C, than below. The change in lipid phase therefore induces large changes in the mobility of the probes attached to the protein that can be followed on the time scale of conventional ESR, i.e. in the range of nanoseconds. Although the spectra evidence extensive rotational averaging in the fluid phase, they contain certain components characteristic of mobility in the slow-motional regime of conventional ESR, i.e. $T_R \sim 10$ ns (e.g. [37]). For comparison, the rotational correlation time of a single transmembrane helix can be estimated from hydrodynamic theory to be in the region of $T_R \sim 110$–220 ns, depending on the value of the intramembranous viscosity in the fluid phase lipid (see e.g. [37]). In the case of an *n*-helix bundle, the rotational correlation time would be *n* times this val-

ue. Presumably, therefore, the spin probes have motion independent of the protein backbone, in the fluid phase of the lipid.

Furthermore, at temperatures in the fluid phase of DPPC, the spectra of the two spin-labelled proteins are significantly different, suggesting intrinsic differences in the rotation mode of the proteins once reconstituted in fluid phospholipid bilayers. These differences indicate that the mobility of the protein-attached spin probe depends, at least partially, on the geometry of the lipid-protein complex in each case, and not just on the intrinsic mobility of the probe itself in the bilayer environment. Differences in the preferential rotational axis of SP-B and SP-C can be inferred from the ESR spectral line shapes of the spin-labelled proteins. The spectrum of TEMPO-SP-C at 45°C is indicative of preferential rotation about the spin label *x*- or *y*-axis, whereas that of TEMPO-SP-B more closely approximates anisotropic line shapes expected for preferential rotation about the orthogonal *z*-axis [38]. This result is compatible with the different modes of insertion proposed for the two proteins in surfactant environments. SP-C has been proposed to form a transmembranal hydrophobic α-helix in phospholipid bilayers [35,39], while SP-B, a protein with amphipathic α-helical motifs, has been suggested to have a peripheral location in the bilayers, with some differences in the extent of membrane penetration depending on the method of reconstitution [8,13,14,40].

3.3. *Effect of temperature on the rotational dynamics of SP-B and SP-C*

Fig. 3 presents the ESR spectra of TEMPO-SP-B or TEMPO-SP-C in DPPC bilayers at different temperatures between 20°C (DPPC gel phase) and 60°C (DPPC fluid phase) including the phospholipid phase transition range. Both proteins show ESR spectra with decreasing outer hyperfine splittings as the temperature increases. The decrease in the outer splitting of the TEMPO-SP-B spectra is apparently continuous and progressive throughout the temperature range studied, while the ESR spectrum of TEMPO-SP-C shows a more abrupt decrease in splitting and coexisting two-component spectra at around 35–39°C, in the region of the phase transition temperature of DPPC.

The temperature profiles of the outer hyperfine splittings of TEMPO-labelled SP-B and SP-C are compared in Fig. 4 with those of spin-labelled phosphatidylcholine probes bearing a reporter spin label either closer to the headgroup, on carbon-5 of the *sn*-

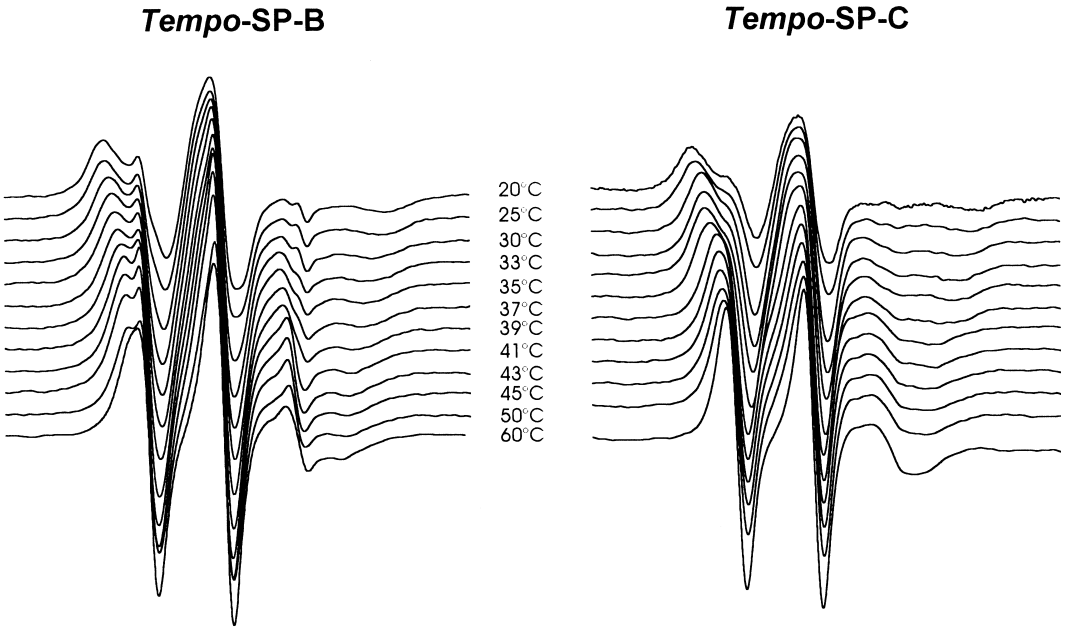

Fig. 3. Temperature dependence of the ESR spectra of TEMPO-SP-B (left) and TEMPO-SP-C (right) reconstituted in bilayers of DPPC (8% protein-to-lipid ratio, w/w). Total scan width, 100 G.

2 acyl chain, or on carbon-12, closer to the bilayer core. As was observed for the spectra of Fig. 3, TEMPO-SP-B shows a progressive increase in mobility with a somewhat steeper gradient at the phase transition of DPPC, which, however, is accompanied by an abrupt decrease in splittings of the spectra of both phospholipid spin probes in bilayers containing a comparable amount of unlabelled SP-B. The temperature profile of TEMPO-SP-C shows a sharp increase in mobility at temperatures close to the phase transition temperature of DPPC. This is accompanied by coexisting spectral components that are characteristic of the two interconverting lipid phases. SP-C therefore partitions into both gel and fluid lipid phases in the phase coexistence region; the situation for SP-B is less clearly differentiated. Effects of the phase transition in the bilayer on the rotational dynamics of several membrane proteins has been already described by other authors. Bacteriorhodopsin [41], rhodopsin [42] and others [34,43] all show a profile of increasing mobility accompanying the gel-to-liquid phase transition of their host membranes. What is more interesting in our experiments is that the temperature profile is qualitatively different for the two proteins studied, SP-B and SP-C, reflecting an intrinsically different structural relationship to the bilayer for the two proteins. The values of A_{max} decrease gradually through the phase transition region for spin-labelled SP-B, whereas those for spin-labelled SP-C decrease abruptly at the phase transition. SP-C, the small, extremely hydrophobic, transmembrane peptide, behaves in terms of rotational dynamics, almost like a lipid molecule. Actually, from this and other studies on lipid-protein interactions with SP-C [10], SP-C could be considered to be the peptide most similar to a lipid molecule. On the other hand, the temperature profile of the spin probe attached to SP-B is probably a consequence of the overall change in membrane dynamics that the protein senses when it is associated with the surface region of the bilayer.

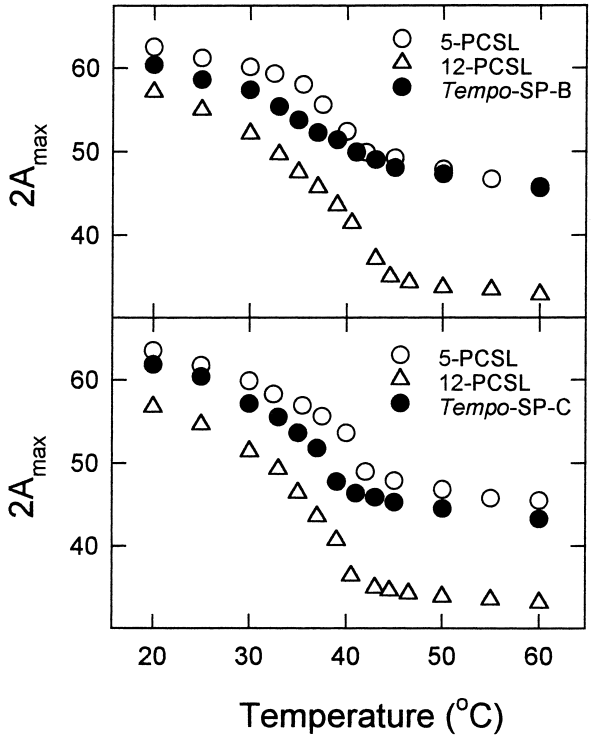

Fig. 4. Temperature dependence of the maximum outer hyperfine splitting, $2A_{max}$, of the spectra of TEMPO-SP-B (upper panel) and TEMPO-SP-C (lower panel) reconstituted in DPPC bilayers (8% protein/lipid ratio, w/w). The temperature profile of the hyperfine splittings of spin-labelled proteins is compared in each panel with those of spin-labelled phosphatidylcholine probes bearing the doxyl group either at position 5 (5-PCSL) or at position 12 (12-PCSL) of the *sn*-2 acyl chain and incorporated in bilayers of DPPC containing 10% (w/w) of the corresponding non-labelled protein.

Interestingly, the outer hyperfine splittings of both TEMPO-SP-B and TEMPO-SP-C begin to show a significant increase at around 36–37°C, which is the temperature at which the pretransition occurs in pure DPPC bilayers [13,44]. This fact suggests that the mobility of the spin-labelled proteins, which is very limited in the 'frozen' gel phase of DPPC bilayers, undergoes a significant qualitative change at temperatures corresponding to those of pretransition region. This structural change in the lipid-protein complex possibly corresponds to changes in the lipid dynamics that are similar to those accompanying the pretransition [45]. In the presence of surfactant proteins, the main transition is broadened to such an extent that distinct pretransitional behaviour is no longer observed (see Fig. 4). A similar behaviour has been reported for other membrane proteins which also show a temperature dependence of their rotational dynamics, with pronounced increases in mobility at temperatures below the phase transition of the host lipid [46].

This behaviour can be particularly significant for the biophysical properties of pulmonary surfactant. It is widely assumed in the surfactant field that

DPPC has been evolutionarily selected as the main tensoactive species of mammalian surfactant because, at the physiological temperature of 37°C, just below the phase transition temperature of DPPC, an interfacial monolayer enriched in DPPC can be compressed to, and sustain, maximal surface pressures (i.e. minimal surface tensions). Although natural surfactant includes other phospholipid components and the simultaneous presence of the different surfactant proteins, the data of the present work suggest that, at temperatures above the pretransition of pure DPPC, i.e. 37°C, the hydrophobic surfactant proteins SP-B and SP-C already acquire substantial rotational mobility, which could be an important feature for the ability of these proteins to modulate the biophysical properties of the tensoactive monolayer which is thought to become preferentially enriched in DPPC due to several mechanisms [47]. SP-B and SP-C, for instance, have been implicated in (i) the insertion of phospholipid molecules at the air-water interface [4,5], (ii) the selective enrichment of the surfactant monolayer in DPPC, under compression [18,19,48], (iii) the stabilization of the DPPC-enriched monolayer at the highest compression rates [5,49], and (iv) the respreading of the monolayer after collapse in successive respiratory cycles [18,19,22]. All these functional effects could be critically dependent on the dynamic features exhibited by these proteins in the narrow temperature range defined from the pretransition to the main phase transition of the host phospholipid.

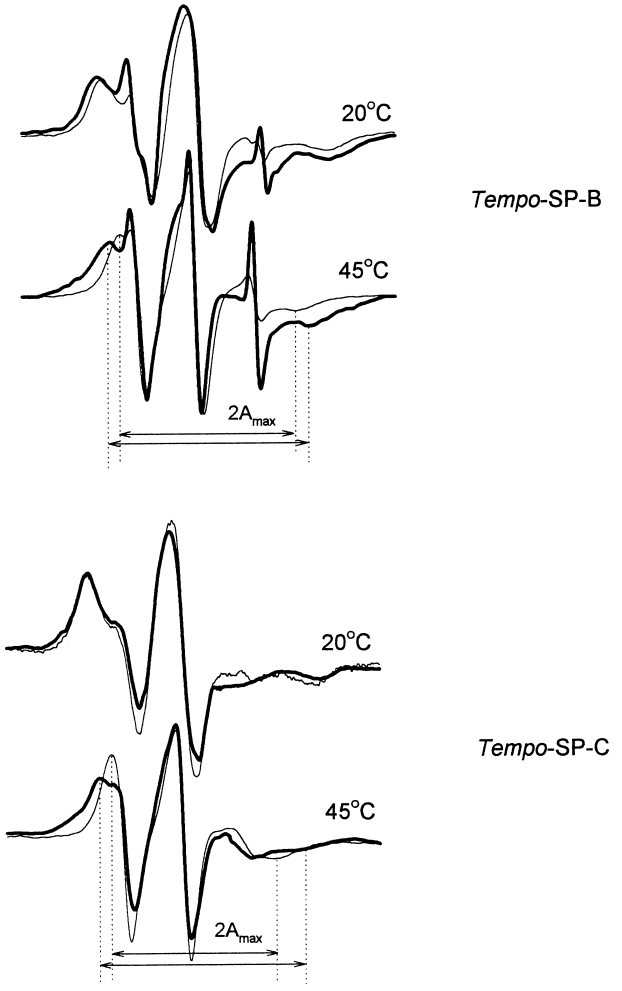

Fig. 5. ESR spectra of TEMPO-labelled SP-B and SP-C reconstituted in DPPG bilayers (bold lines) compared with the spectra of the same spin-labelled proteins in DPPC (thin lines), at temperatures below (20°C) and above (45°C) the phase transition temperature of both phospholipids. Protein-to-lipid ratio in the samples was 8% (w/w); total scan width, 100 G.

3.4. Rotational dynamics of SP-B and SP-C in DPPG bilayers

Phosphatidylglycerol (PG) is the second main phospholipid class in surfactant. It has been proposed that electrostatic interactions between this acidic phospholipid and the positively charged hydrophobic surfactant proteins SP-B and SP-C could mediate at least some of the effects of these proteins on surfactant phospholipids. We have reported, in a recent study using spin-labelled phospholipids, that SP-B interacts selectively with PG over other acidic phospholipids under conditions of physiological ionic strength [10].

The effect of possible specific interactions between PG and hydrophobic surfactant proteins on the rotational dynamics of spin-labelled SP-B or SP-C has been explored here. Fig. 5 gives the ESR spectrum of TEMPO-SP-B and TEMPO-SP-C reconstituted in bilayers of DPPG at temperatures below (20°C) and above (45°C) the phase transition temperature of the lipid (41°C), compared with the spectra of the same proteins in DPPC. The spectra of either spin-labelled protein in DPPG are qualitatively similar to those in DPPC at the same temperature in the gel phase of the lipid. In both host lipid gel phases, the spectra are in the slow-motion regime. The outer hyperfine splittings are, however, consistently higher in DPPG than in DPPC. This could indicate an even stronger

motional restriction of the proteins in the gel phase of DPPG, although contributions to the increase in A_{max} may also arise from the electrostatic field at the lipid surface [29]. When the ESR spectra of the proteins in DPPC and DPPG are compared at temperatures above the phase transition of the lipid, the spectral differences are considerably greater than are those in the gel phase. The outer splittings of the ESR spectra of the proteins reconstituted in DPPG bilayers are always larger than those of the proteins in DPPC (see also Table 1). This indicates that the interactions between SP-B or SP-C and the negatively charged DPPG membrane surface cause additional immobilization or reorientation of the spin probes. This differential behaviour probably originates in electrostatic lipid-protein interactions between DPPG headgroups and the protein segments where the spin labels are attached, possibly with accompanying conformational changes. In both SP-B and SP-C, the N-terminal segment has, or is flanked by, positively charged regions which could tend to anchor the spin-labelled N-terminal end of the proteins to the DPPG bilayer surface, therefore causing an additional perturbation of the mobility of the probe.

The fine conformational modulation of the small polar portions of the hydrophobic surfactant proteins in PG-containing bilayers or monolayers could mediate lipid-protein or protein-protein interactions essential for their structure-functional competence.

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