Biochimica et Biophysica Acta 813 (1985) 174–182
Elsevier

BBA 72473

Strong interactions between a spin-labeled cholesterol analog and erythrocyte proteins in the human erythrocyte membrane

Michel Seigneuret *, Edith Favre, Gil Morrot and Philippe F. Devaux

Institut de Biologie Physico-Chimique, 13, rue Pierre et Marie Curie, 75005 Paris (France)

(Received September 12th, 1984)

Key words: Cholesterol; Band 3; Lipid-protein interaction; Spin label; ESR; (Erythrocyte membrane)

We have used a spin label analog of cholesterol bearing a nitroxide on the alkyl chain (26-nor-25-doxylcholestanol) to study cholesterol-protein interactions in the human erythrocyte membrane. As judged from the ESR spectrum, the spin label is readily incorporated into the membrane when added from a concentrated ethanolic solution to a cell or ghost suspension. With intact erythrocytes or white ghosts in isotonic buffer, the ESR spectrum is a superposition of a mobile component and a strongly immobilized component (outer hyperfine splitting 61–63 G). The latter corresponds to approx. 45% of the signal, a percentage which is barely affected by varying the temperature between 5 and 37°C. Removal of the cytoskeletal proteins spectrin and actin by low ionic strength treatment or of all extrinsic proteins by alkali treatment of ghosts reduces the immobilized fraction to approx. 25%. The effect of controlled proteolysis of intrinsic proteins was also tested. Pre-treatment of cells with chymotrypsin or pre-treatment of unsealed ghosts with trypsin has no effect on the ESR spectrum obtained with alkali-treated membranes. On the other hand, after chymotrypsin treatment of unsealed ghost, which reduces the band 3 protein to a 17.5 kDa membrane fragment, the strongly immobilized component is no longer observable. These data show that the cholesterol analog 26-nor-25-doxylcholestanol interacts strongly with one or several proteins of the erythrocyte membrane. That the intrinsic protein band 3 is involved is suggested by the disappearance of the immobilized fraction occurring upon chymotrypsin digestion of this protein. Our results are thus consistent with the proposal of a selective cholesterol-band 3 interaction in the erythrocyte membrane (Schubert, D. and Boss, K. (1982) FEBS Lett. 150, 4–8). Our data also suggest that this interaction is influenced by cytoskeletal proteins, an effect which can be explained considering the known linking of band 3 to the erythrocyte cytoskeleton via ankyrin. Experiments have also been carried out with 3-doxylandrostanol, a more commonly used cholesterol spin-label analog. With this spin label, at all temperatures investigated, we found it impossible to demonstrate unambiguously the existence of two separate spectral components. It is suggested that 26-nor-25-doxylcholestanol is a better reporter of cholesterol behavior in membranes.

Introduction

Cholesterol is a major lipid constituent of many eukaryotic plasma membranes. There is a vast literature concerning the influence of cholesterol upon lipid packing and mobility in both model and biological membranes (for review see Refs. 1, 2). Emphasis has been put on cholesterol-phospholipid interactoins rather than on cholesterol-protein interactions. Accordingly, a partial view of the biological role of cholesterol is that of a regulating agent of plasma membrane fluidity [1,2].

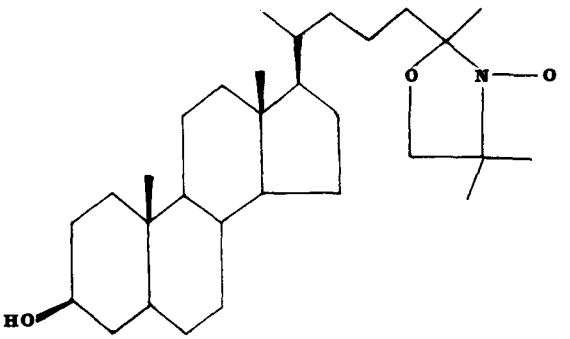
* Present address: Centre d'Etudes Nucléaires de Saclay, Départment de Biologie, Service de Biophysique, 91191 Gif-sur-Yvette Cedex, France.

However, there is increasing evidence that the lateral or transverse distribution of cholesterol is not uniform in many membranes [3–5]. In particular, in the case of the erythrocyte membrane, a transbilayer asymmetry of cholesterol has been suggested [6,7]. Such asymmetry may involve cholesterol-protein interactions. Also in erythrocyte, Cherry and collaborators have shown that the membrane cholesterol level can modulate the aggregation state of the intrinsic protein band 3, provided that interactions with the cytoskeleton have been removed [8,9]. As a possible explanation, these authors suggested that cholesterol may be trapped within band 3 aggregates. As a matter of fact, a strong and preferential interaction of solubilized band 3 with cholesterol has been evidenced in monolayers [10,11] though no direct proof exists in the case of native membrane.

Electron spin resonance (ESR) is an efficient technique for the detection of the specificity of lipid-protein interactions (for reviews see Refs. 12 and 13). The advantages of ESR over NMR are a better-suited time-scale for the detection of lipids at the protein boundary and a higher sensitivity. On the other hand, a possible drawback of ESR is the necessity of using spin-labeled molecules. A nitroxide probe may represent a perturbation, the importance of which is difficult to predict. In the case of phospholipids, spin labeling of the β-chain has recently been shown to have no influence upon the affinities for intrinsic proteins [12,13]. Spin-labeled analogs of androstane and androstanol have several times been used as reporters of sterol-protein interactions in membranes [14,15]. However, in this case, the results may be questionable, since these molecules bear little resemblance to cholesterol due to the perturbed head-group and the absence of a side-chain. Other labels which are more likely to mimic cholesterol have been synthesized [16–18] but few applications to the study of lipid-protein interactions have been performed [19].

In the present study we have used 26-nor-25-doxylcholestanol, a cholesterol analog spin-labeled on the side-chain. This spin label is referred to in the text as 25-doxylcholestanol.

This label was first used by Suckling and Boyd [17] in pure lipid membranes. Here we have incorporated 25-doxylcholestanol in human erythrocytes and derived membranes. Lipid-protein interactions were evaluated by quantitation of the broad 'strongly immobilized' component in ESR spectra. Since erythrocyte membranes contain several major polypeptides, we have attempted to identify the proteins involved by selective extraction or controlled proteolysis. This allowed us to obtain evidence of the role of the intrinsic protein band 3.



Scheme I. 26-Nor-25-doxylcholestanol.

Materials and Methods

Preparation of cells and membranes. Erythrocytes from less than 5-day-old human blood drawn on citrate/phosphate/dextrose were washed five times with a 145 mM NaCl/5 mM KCl/1 mM $MgSO_4$/10 mM glucose/20 mM Hepes buffer, pH 7.4 (buffer A). Proteolysis of cells was obtained by incubation at a 25% hematocrit in buffer A containing 1 mg/ml chymotrypsin (Sigma, *N*-α-*p*-tosyl-L-lysine chloromethyl ketone treated) for 60 min at 37°C. The reaction was stopped by cooling the cells and extensive washing.

For membrane preparations, all buffers contained 0.1 mM PMSF and 5 μg/ml pepstatin A except when proteolysis was to be performed. White unsealed ghosts were prepared according to Ref. 20 except that a 1 mM EDTA/10 mM sodium phosphate buffer, pH 8 (buffer B) was used for hemolysis and washing. Removal of spectrin and actin was obtained by incubating ghosts in 40 vol. 0.1 mM EDTA (pH 8.5) for 30 min at 37°C. For complete extrinsic protein extraction, ghosts were incubated in 40 vol. 1 mM EDTA (pH 12) for 5 min at 0°C. Two-dimensional thin layer chromatography of the extracted lipids was performed before and after pH 12 treatment: chloroform/methanol/acetic acid/water (60 : 30 : 8 : 2.85) and

chloroform/methanol/25% ammonium hydroxide/water (90 : 54 : 5.7 : 5.3) respectively. No formation of lyso derivatives could be detected.

In both cases incubation was followed by pelleting and washing in buffer B. Proteolysis of ghosts was carried out by their incubation (2 mg protein/ml) in buffer B containing either 2.5 mg/ml chymotrypsin or 0.25 mg/ml trypsin (Sigma, L-1-tosylamide-2-phenylethyl chloromethyl ketone-treated) for 60 min at 37°C. The reaction was stopped by addition of 0.1 mM phenylmethylsulfonyl fluoride followed by extensive washing. Ultimately, all membranes were resuspended in a 100 mM KCl/2 mM $MgSO_4$/1 mM EGTA/20 mM sodium phosphate buffer (pH 7.4).

Analytical procedures. Protein extractions and proteolysis were checked by SDS slab gel electrophoresis performed according to Laemmli [21] with a 4% acrylamide stacking gel and a 7.5–15% acrylamide gradient resolving gel. Gels were stained for proteins and sialoglycoproteins using standard procedures [20]. For molecular weight calibration, a standard protein mixture composed of 10 proteins ranging from 14.2 to 205 kDa (Sigma) was electrophoresed with the samples. Proteins were determined according to Lowry et al. [22] using bovine serum albumin as a standard. Lipid phosphorus was assayed according to Rouser et al. [23].

Spin labeling and ESR experiments. 26-Nor-25-doxylcholestanol was synthesized according to Refs. 16 and 17. The 3-doxyl androstanol was purchased from Aldrich Company. The spin label was introduced into the cell suspension (25% hematocrit) or membrane suspension (1 mg/ml) from an ethanol solution (final proportion of ethanol < 1%), followed by gentle homogenization, incubation at 37°C for 5 min and pelleting. Incorporation was complete as judged from the disappearance of the broad ESR line characteristic of the aggregated label in water. The final spin-label concentration corresponded to 1% of total phospholipids. ESR spectra were recorded with a Varian E 109 spectrometer interfaced to a Tektronix 4051 computer and equipped with a temperature-control system. Quantitation of the fraction of 'strongly immobilized component' in experimental spectra was performed by subtraction of a computer-simulated spectrum corresponding to restricted motion. Details of such procedures have been published elsewhere [12,13,24,25]. The method used for simulation is described in Ref. 26. Ascorbate reduction experiments were carried out according to Ref. 27.

Results

ESR spectra of 25-doxylcholestanol in intact erythrocyte membranes

ESR spectra of 25-doxylcholestanol incorporated in freshly prepared human erythrocyte ghosts are shown in Fig. 1 as a function of temperature. Identical spectra are obtained with intact erythrocytes (not shown). Two components are present at all temperatures. The narrow component is characterized by a double minimum at high field, a feature which always seems to occur for freely diffusing 25-doxylcholestanol in membranes [17]. The broad component possesses a very large outer hyperfine splitting which only weakly varies with temperature, from 63.5 G at 5°C to 61 G at 37°C. This indicates a strong immobilization of a fraction of the probe at the ESR time-scale (i.e. $\tau_c \geqslant 10^{-7}$ s) at all temperatures.

In order to estimate this immobilized fraction, spectral subtractions were performed using a computer-simulated ESR spectrum corresponding to the broad component. The same calculated spectrum was used at all temperatures, a procedure

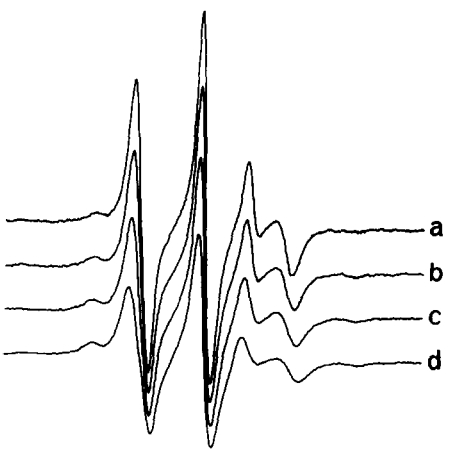


Fig. 1. ESR spectra of 25-doxylcholestanol as a function of temperature in intact erythrocyte ghosts. (a) 37°C; (b) 25°C; (c) 15°C; (d) 5°C. Scan range, 100 G.

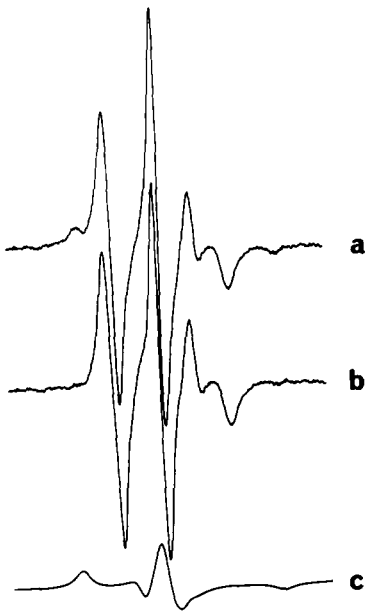


Fig. 2. Example of spectral subtraction used to quantify the amount of immobilized component in spectra of 25-doxylcholestanol in erythrocyte membranes. (a) ESR spectrum of 25-doxylcholestanol in intact erythrocytes at 5°C; (b) narrow ESR component yielded by spectral subtraction; (c) simulated ESR spectrum used for spectral subtraction. The latter spectrum was simulated according to Ref. 26 using the following parameters: $T'_{\parallel} = 31.4$ G. $T'_{\perp} = 7.1$ G. $\Delta H = 5.4$ G. $LW(+1) = 3.6$ G. $LW(0) = 3.9$ G, and $LW(-1) = 4.5$ G. The subtraction was judged adequate when the spectral contribution of the broad component was properly canceled. The corresponding percentage of immobile component was $48 \pm 5\%$.

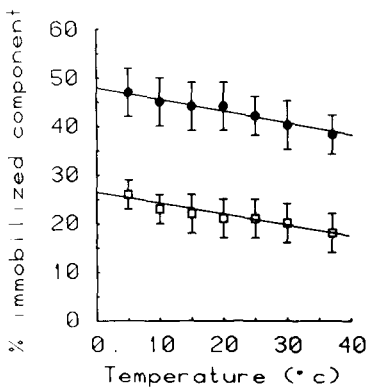


Fig. 3. Temperature dependence of the percentage of immobilized component of 25-doxylcholestanol in intact erythrocyte ghosts (filled circles) and alkali-treated ghosts (open squares). For each point, the percentage was determined by spectral subtraction performed as depicted in Fig. 2. An identical simulated broad spectrum was used in each case (see text for discussion). The error bars correspond to the range of subtractable amount of the simulated spectrum yielding a pure mobile spectrum.

which is justified by the weak temperature-dependence of the broad component outer splitting. An example of spectral subtraction is shown in Fig. 2 and the result of the quantification is plotted vs. temperature in Fig. 3. The amount of immobilized component is of the order of 40–50% and exhibits only weak temperature-dependence. This small temperature-dependence may be only apparent, considering the fact that the lineshape of the 'true' broad component may be slightly temperature-dependent.

Effect of removal of extrinsic proteins

The ESR spectra of 25-doxylcholestanol incorporated in erythrocyte membranes freed of all extrinsic proteins by alkali treatment are shown in Fig. 4 as a function of temperature. Identical spectra are obtained if only spectrin and actin are extracted by low ionic strength treatment. Comparing these spectra to those of Fig. 1, two distinct modifications can be noticed: (1) line-broadening of the mobile component, (2) amplitude decrease of the immobilized component. The outer splitting of the immobilized component could be precisely measured only at 5°C and was 63.5 G, which is

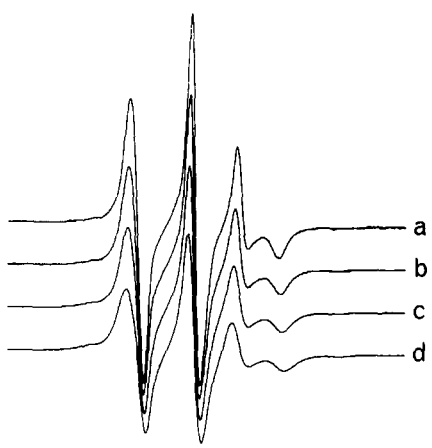


Fig. 4. ESR spectra of 25-doxylcholestanol as a function of temperature in alkali-treated erythrocyte membranes. (a) 37°C; (b) 25°C; (c) 15°C; (d) 5°C. Scan range, 100 G.

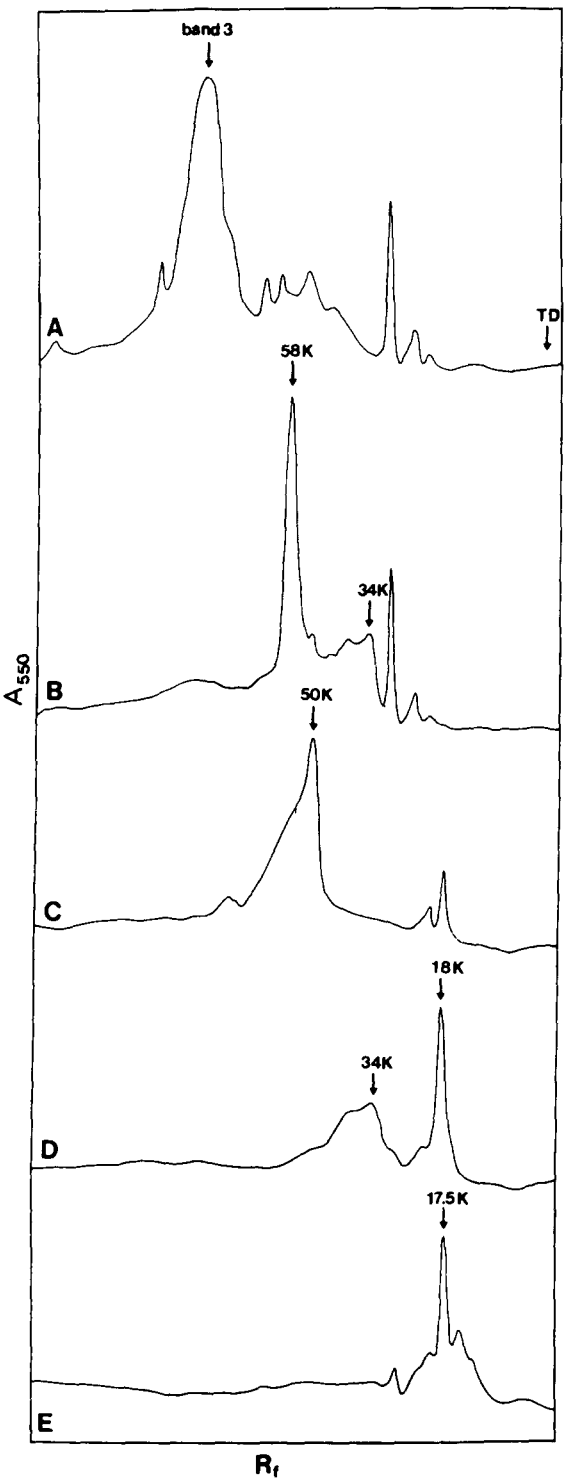


Fig. 5. Scans of Coomassie blue-stained SDS-polyacrylamide electrophoresis gels of alkali-treated erythrocyte membranes submitted to various proteolytic pre-treatments prior to alkaline extraction. (a) no pretreatment; (b) membranes derived from chymotrypsin-pretreated cells; (c) membranes derived from trypsin-pretreated ghosts; (d) membranes derived from trypsin-pretreated ghosts originating from chymotrypsin-pretreated cells; (e) membranes derived from chymotrypsin-pretreated ghosts. Details are given in Materials and Methods. TD, tracking dye; K, kilodaltons.

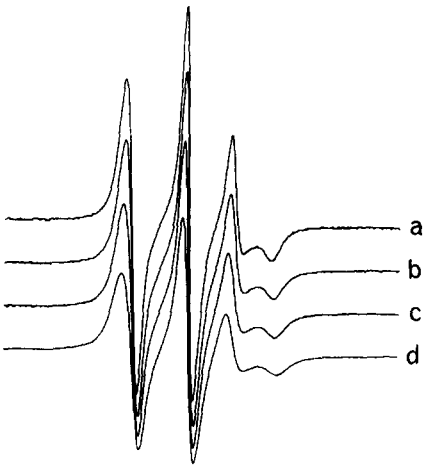


Fig. 6. ESR spectra of 25-doxylcholestanol as a function of temperature in alkali-treated erythrocyte membranes pre-treated with chymotrypsin prior to alkaline extraction. (a) 37°C; (b) 25°C; (c) 15°C; (d) 5°C. Scan range, 100 G.

the value obtained in cells and ghosts. Spectral subtraction indicates that the amount of broad component is reduced to approximately half its initial value upon extrinsic protein removal. This is true at all temperatures (Fig. 3).

Effect of proteolysis of intrinsic proteins

Since a significant amount of broad component is resistant to extrinsic protein extraction, it is likely to be related to interaction with intrinsic proteins. ESR spectra of 25-doxylcholestanol were then recorded in membrane submitted to various proteolytic treatments prior to alkaline extraction. Several treatments had no effect on the ESR spectra of alkali-treated membranes as compared to those in Fig. 4. These included chymotrypsin treatment of intact cells, which cleaved band 3 into two membrane-bound 58 K and 34 K fragments (Fig. 5, gel B), trypsin treatment of ghosts which re-

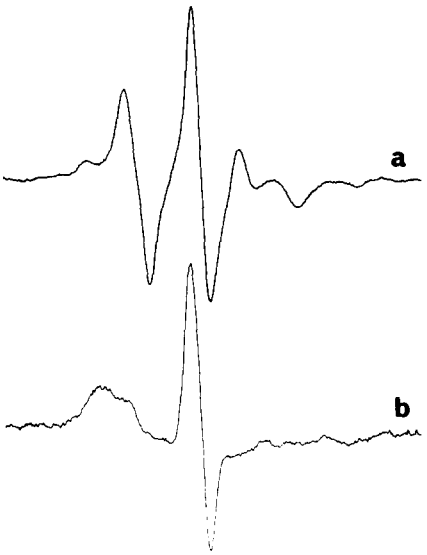
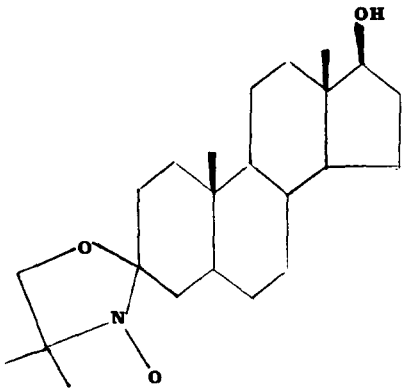


Fig. 7. ESR spectra of 25-doxylcholestanol (a) and 3-doxylandrostanol (b) at 5°C in intact erythrocytes. Scan range, 100 G.

moved the soluble cytoplasmic band 3 fragment and produced a 50 K membrane bound fragment (Fig. 5, gel C) as well as the sequential combination of both treatment which left only 18 and 34-kDa fragments in the membrane (Fig. 5, gel D). On the other hand, chymotrypsin treatment of ghosts (at low ionic strength), which reduced band 3 to a 17.5 kDa membrane fragment (Fig. 5, gel E), had a dramatic effect *. As can be seen in Fig. 6, the immobilized component was no longer observable in such proteolysed and alkaline-treated membranes **. The sialoglycoprotein glycophorin A was cleaved by all proteolytic treatments (not shown).



Scheme II. 3-Doxylandrostanol.

Comparison of 26-nor-25-doxylcholestanol and 3-doxylandrostanol in erythrocyte membranes

The behaviour of 25-doxylcholestanol spin label was compared to that of 3-doxylandrostanol, a more frequently used cholesterol analog (Scheme II). Spectra of both labels at 5°C in the membrane of intact erythrocytes are shown in Fig. 7. The overall spectrum of 3-doxylandrostanol is much more motionally restricted than that of 25-doxylcholestanol. On the other hand, the former spectrum does not allow the decomposition into two distinct components. This was true at all temperatures between 5 and 37°C. Of course it is always possible to decompose an ESR spectrum into several components if one chooses two very similar components. But this is totally arbitrary and would yield no useful information.

The two probes were also compared with regard to their ascorbate accessibility in the erythrocyte membrane. While the androstanol derivative was reduced very rapidly and completely by 10 mM ascorbate (half-time approx. 5 min), 25-doxylcholestanol yielded only a 10% signal decrease in 2 h (not shown).

Discussion

Spin-labeled lipids have been used by many authors to study the specificity of lipid-protein interactions in membranes. Lipids at the protein

* Apparent molecular weights reported here for the proteolytic segments of band 3 slightly differ from those given in the literature [28–30]. This is likely to be due to the different electrophoresis conditions used. Chymotrypsin treatment of unsealed ghosts at low ionic strength also produces a 9 kDa fragment from band 3 [30]. However, its recovery on gels is variable [28]. The broad featureless band underlying the 17.5 kDa band in Fig. 5 (gel E) may possibly account for this fragment.

** It may be asked why intersubtraction between spectra of Fig. 4 and Fig. 6 was not used to estimate the amount of broad component. However, as can be seen from careful comparison of the spectra, subtle changes in the lineshape of the mobile component occur together with the disappearance of the immobilized component. Thus, since the percentage of the latter component is weak in spectra of Fig. 4, pairwise subtraction cannot be used.

boundary are revealed by the presence of broad ESR lines (low mobility), while lipids in a fluid bilayer give rise to a narrow signal. Quantitation of the two components allows one to estimate lipid-protein affinity [12,13,31]. This report represents the first study using the 25-doxylcholestanol spin probe in a biological membrane. This spin label can be considered a priori as a better cholesterol analog than the usual androstane or androstanol derivatives. It possesses the 3-β-hydroxyl residue as well as a side-chain, which are both necessary for the sterol to exert its lipid ordering effect [1]. On the other hand, the intracyclic double bond, which is absent in 25-doxylcholestanol, is of little importance for that [1]. The question may arise as to whether the presence of the nitroxide group may be perturbing. According to ascorbate reduction, the paramagnetic group of 25-doxylcholestanol is not accessible from the aqueous phase, suggesting that the spin-labeled side-chain is deeply embedded in the bilayer as the side-chain of cholesterol. An influence of the nitroxide group on sterol-protein interactions appears unlikely. In the case of phospholipids, recent studies strongly suggest that the presence of a nitroxide on the acyl chain does not modify the specificity of interaction with a particular membrane protein [12,13,31]. It may also be mentioned that the addition of a nitroxide to the acyl chain of acyl-CoA does not affect its strong affinity for the ADP-ATP carrier in mitochondrial inner membrane [32]. Finally it is noteworthy that the ESR spectrum of 25-doxylcholestanol in acetylcholine receptor-rich membranes from *Torpedo marmorata* is characterized by a single narrow component (J.F. Bureau, personnal communication), so that the immobilization observed here appears specific to the erythrocyte membrane.

Being located on a flexible chain, the nitroxide group of 25-doxylcholestanol gives rise to narrow ESR lines when diffusing freely in a lipid bilayer. Thus, any interaction with a rigid protein should drastically modify the lineshape. On the other hand, androstane or cholestane spin labels intrinsically have a very hindered motion, so that further immobilization may be more difficult to detect, though it has been performed successfully in other systems [12,14,15,18,24]. This, as well as structural perturbations of the head group, may explain why a two-component spectrum is not obtained with 3-doxylandrostanol in the erythrocyte membrane.

So far, most ESR studies on lipid-protein interactions have involved reconstituted membranes containing a single protein species. Low lipid-to-protein ratios are often used in order to enhance the spectral contribution of the boundary lipids. Nevertheless, at high temperatures, the quantitation of the broad (protein-bound) component is difficult, since the latter tends to collapse with the narrow (bilayer) component. This could be due to rapid exchange of spin-labeled lipids between the two environments [26]. Another explanation is that the stronger temperature-dependence of the narrow component may cause the broad component to be outweighted.

On the other hand, the present study indicates that a large fraction of the cholesterol analog 25-doxylcholestanol interacts strongly with one or several proteins in the intact human erythrocyte membrane at temperatures ranging from 5 to 37°C. The large immobilized component observed cannot be attributed to simple nonspecific contact of the spin-labeled chain with the boundary of intrinsic proteins. As a matter of fact, the weak temperature-dependence of both the percentage and the outer hyperfine splitting of this component indicates that the 25-doxylcholestanol label exchanges slowly between the protein and the lipid bilayer environment on the ESR time-scale. This type of spin-label data is reminiscent of what was previously found for covalently linked long-chain spin labels trapped within oligomeric or aggregated intrinsic proteins such as the Ca^{2+}-dependent ATPase from sarcoplasmic reticulum [33] or erythrocyte band 3 [34]. Thus, it is tempting to interpret the large immobilized component observed for 25-doxylcholestanol in erythrocyte membranes as arising from label trapped between intrinsic proteins. Indeed, band 3, the major erythrocyte protein, may be a dimer or a tetramer (for review see Ref. 35) and even more extensive states of association have been suggested [9]. Furthermore, our results do suggest that band 3 is the principal protein involved in 25-doxylcholestanol immobilization. The broad ESR component partially resists extraction of extrinsic proteins but disappears after chymotrypsin digestion of unsealed ghosts, a treatment known to extensively

cleave and inactivate band 3 [30]. Less extensive proteolysis which preserves the anion-transport portion of band 3 is ineffective, although other instrinsic proteins are cleaved.

This strong 25-doxylcholestanol band 3 interaction implies very high specificity of the cholesterol analog for the protein. Indeed, spin-labeled phospholipids or fatty acids do not give rise to such a strongly immobilized component in the erythrocyte membrane [26,37]. Schubert and collaborators suggested from monolayer studies with the solubilized protein that a specific 1–1 association could occur between band 3 and cholesterol [10,11]. Our data partially agree with their results in suggesting a selective interaction between cholesterol and band 3. On the other hand, while the discrete 1–1 association postulated by these authors may contribute to the immobilized component observed with 25-doxylcholestanol, it cannot account for its total percentage. Indeed, the immobilized fraction is respectively 40% and 19% in intact and alkali-treated membranes at 37°C. This would correspond to approx. 74 and 35 immobilized cholesterol molecules per band 3 monomer *. The explanation we propose is that small domains of aggregated band 3 exist in the erythrocyte membrane, in which proteins are separated by one or two lipid layers specifically enriched in cholesterol. Cholesterol itself could contribute to the formation and the stabilization of these domains, since this lipid has been suggested to be non-randomly distributed in bilayers [40] and has been shown to induce aggregation of intrinsic proteins in reconstituted membranes [41,42]. Thus, the spectral characteristics and weak temperature-dependence of the immobilized component observed with 25-doxylcholestanol as well as its stoichiometry relative to band 3 argue in favor of a selective trapping of cholesterol inside band 3 aggregates.

Our results also show that extraction of spectrin and actin or of all extrinsic proteins decreases approximately by half the amount of immobilized 25-doxylcholestanol. It appears unlikely that this reflects a direct interaction between the cholesterol analog and some extrinsic protein. Rather, it may be that the spectrin-actin network, by controlling the lateral distribution and aggregation of intrinsic proteins, as suggested [43], also modulates the extent of cholesterol trapping within band 3 aggregates. It is well known that a subpopulation of band 3 is linked to the cytoskeleton via ankyrin and is thereby not free to diffuse (for review see Ref. 44).

The possibility that cholesterol could be trapped within band 3 aggregates was previously suggested by Mühlebach and Cherry [9]. These authors found that cholesterol could affect the rotational diffusion of band 3 only after removal of interactions with the cytoskeleton. Thus both their results and the present report suggest a strong interconnection of cytoskeletal and cholesterol-protein interactions in controlling the lateral distribution of band 3.

Finally, a comment is required concerning two recent reports which may seem to conflict with our results. Kelusky et al. [45] have shown that a deuterated cholesterol molecule yielded nearly identical ^{2}H-NMR spectra in erythrocyte membranes and in corresponding extracted lipids. However, considering the low signal-to-noise ratio of their spectra, the presence of a broad component in the membrane spectrum cannot be ruled out. Golan et al. [46] have compared the lateral diffusion coefficient of fluorescent analogs of phospholipids and cholesterol in erythrocyte membranes using fluorescence photobleaching recovery and found identical values. However, the possibility cannot be excluded that a fraction of the probes does not diffuse during the time-scale of the experiment. In any case, it must be stressed that NMR and photobleaching have much slower time-scales than ESR. Thus, a lipid may be exchanging rapidly between protein and lipid environment when viewed through the two former methods and nevertheless appear immobilized in an ESR experiment.

Recently a spin-labeled analog of cholesterol, 26-nor-25-doxylcholesterol was synthesized by Dr. A. Bienvenüe. Preliminary experiments show that this spin label, which is even closer to cholesterol than the one used in the present study, behaves very similarly in human erythrocyte membranes, i.e. shows a strong indication of protein binding. (A. Bienvenüe, personal communication.)

* This estimation assumes a molecular weight of 95000 [28] and a weight percentage relative to total protein of 30% [20] for band 3, a cholesterol-to-phospholipid mole ratio of 0.8 [38] and a phospholipid-to-protein weight ratio of 0.66 in the intact membrane [39].

Acknowledgements

This work was supported by grants from the Centre National de la Recherche Scientifique (UA526), the Ministère de l'Industrie et de la Recherche, the Université Paris VII and the Fondation pour la Recherche Médicale.

References

1 Demel, R.A. and De Kruijff, B. (1976) Biochim. Biophys. Acta 457, 109–132
2 Quinn, P.J. (1981) Prog. Biophys. Mol. Biol. 38, 1–104
3 Hoover, R.L., Dawidowicz, E.A., Robinson, J.M. and Karnovsky, M.J. (1983) J. Cell. Biol. 97, 73–80
4 Zagyansky, Y. and Jard, S. (1981) Exp. Cell Res. 132, 387–397
5 Bittman, R. (1978) Lipids 13, 686–696
6 Fisher, K.A. (1976) Proc. Natl. Acad. Sci. USA 73, 173–177
7 Hale, J.E. and Schroeder, F. (1982) Eur. J. Biochem. 122, 649–661
8 Nigg, E.A. and Cherry, R.J. (1979) Biochemistry 18, 3457–3464
9 Mühlebach, T. and Cherry, R.J. (1982) Biochemistry 21, 4225–4228
10 Klappauf, E. and Schubert, D. (1977) FEBS Lett. 80, 423–425
11 Schubert, D. and Boss, K. (1982) FEBS Lett. 150, 4–8
12 Marsh, D. and Watts, A. (1982) in Lipid-Protein Interactions, Vol. 2 (Jost, P.C. and Griffith, O.H., eds.), pp. 53–148, John Wiley, New York
13 Devaux, P.F. and Seigneuret, M., Biochim. Biophys. Acta, in the press
14 Ellena, J.F., Blazing, M.A. and McNamee, M.G. (1983) Biochemistry 22, 5523–5535
15 Brophy, P.J., Horvath, L.I. and Marsh, D. (1984) Biochemistry 23, 860–865
16 Keana, J.F.W., Keana, S.B. and Beetham, D. (1967) J. Am. Chem. Soc. 89, 3055–3056
17 Suckling, K.E. and Boyd, G.S. (1976) Biochim. Biophys. Acta 436, 295–300
18 Keana, J.F.W., Tamura, T., McMillen, D.A. and Jost, P.C. (1981) J. Am. Chem. Soc. 103, 4904–4912
19 Silvius, J.R., McMillen, D.A., Saley, N.D., Jost, P.C. and Griffith, O.H. (1984) Biochemistry 23, 538–547
20 Fairbanks, G., Steck, T.L. and Wallach, D.F.H. (1971) Biochemistry 10, 2606–2617
21 Laemmli, U.K. (1970) Nature (London) 277, 680–685
22 Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265–275
23 Rouser, G., Fleischer, S. and Yamamoto, A. (1969) Lipids 5, 494–496
24 Watts, A., Volotovski, I.D. and Marsh, D. (1979) Biochemistry 18, 5006–5012
25 Devaux, P.F. (1983) in Biological Magnetic Resonance, Vol. 5 (Berliner, L.J. and Reuben, J., eds.), pp. 183–299, Plenum Press, New York
26 Davoust, J. and Devaux, P.F. (1982) J. Magn. Resonance 48, 475–494
27 Seigneuret, M. and Devaux, P.F. (1984) Proc. Natl. Acad. Sci. USA 81, 3751–3755
28 Steck, T.L., Ramos, B. and Strapazon, E. (1976) Biochemistry 15, 1154–1161
29 Ramjeesingh, M., Grinstein, S. and Rothstein, A. (1980) J. Membrane Biol. 57, 95–102
30 Dulze, A.M. and Rothstein, A. (1981) Biochim. Biophys. Acta 646, 471–478
31 Brotherus, J.R., Griffith, O.H., Brotherus, M.O., Jost, P.C. and Silvius, J.R. (1981) Biochemistry 20, 5261–5267
32 Devaux, P.F. Bienvenüe, A., Lauquin, G., Brisson, A.D., Vignais, P.M. and Vignais, P.V. (1975) Biochemistry 14, 1272–1280
33 Andersen, J.P., Fellman, P., Moller, J.V. and Devaux, P.F. (1981) Biochemistry 20, 4928–4936
34 Bittman, R., Sakaki, T., Tsuji, A., Devaux, P.F. and Ohnishi, S. (1984) Biochim. Biophys. Acta 769, 85–95
35 Jennings, M.L. (1984) J. Membrane Biol. 80, 105–117
36 Tanaka, K.I. and Ohnishi, S. (1976) Biochim. Biophys. Acta 426, 218–231
37 Butterfield, D.A. (1982) in Biological Magnetic Resonance, Vol. 4 (Berliner, L.J. and Reuben, J., eds.), pp. 1–78, Plenum Press, New York
38 Lange, Y., Cutler, H.B. and Steck, T.L. (1980) J. Biol. Chem. 255, 9331–9337
39 Rosenberg, S.A. and Guidotti, G. (1969) in Red Cell Membrane – Structure and Function (Jamieson, G.A. and Greenwald, T.J., eds.), pp. 93–109, Lippincott, Philadelphia
40 Copeland, B.R. and McConnell, H.M. (1980) Biochim. Biophys. Acta 599, 95–109
41 Kleemann, W. and McConnell, H.M. (1976) Biochim. Biophys. Acta 419, 206–222
42 Cherry, R.J., Müller, U., Holenstein, C. and Heyn, M.P. (1980) Biochim. Biophys. Acta 596, 145–151
43 Shotton, D., Thompson, K., Wolsy, L. and Branton, D. (1978) J. Cell. Biol. 76, 512–531
44 Haest, C.W.M. (1982) Biochim. Biophys. Acta 694, 331–352
45 Kelusky, E.C., Dufourc, E.J. and Smith, I.C.P. (1983) Biochim. Biophys. Acta 735, 302–304
46 Golan, D.E., Alecio, M.R., Veatch, W.R. and Rando, R.R. (1984) Biochemistry 23, 332–339