Structure and topography of the membrane-binding C2 domain of factor VIII in the presence of dodecylphosphocholine micelles

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A 21 residue peptide from the C2 domain of the antihemophilic factor VIII competes with factor VIII for membrane-binding sites *in vitro*. Here, we provide the structure and topography of the peptide in solution, on dodecylphosphocholine (DPC) micelles, determined using ¹H-NMR spectroscopy. The peptide assumes an amphipathic structure comprising an extended N-terminal region and a C-terminal helix. The average root-mean-square deviation is 0.7 ± 0.2 Å for the superimposition of the backbone atoms of Ile⁶ to Arg¹⁸ on the lowest energy structure. Whereas the backbone conformation is similar to that in SDS micelles, the Trp¹¹ side-chain orientation is dramatically changed. The indole ring is nearly parallel to the peptide backbone in SDS micelles but perpendicular in DPC micelles. Further, p*K*_a values of the two histidines change by more than 1 pH unit in SDS relative to DPC, which localizes the imidazole rings to the interfacial region. Line-broadening induced by spin-labelled

phosphatidylcholine shows that most of the amino acid side-chains that penetrate the DPC micelle are hydrophobic. Thus, the long axis of the peptide lies parallel to the micelle surface and the hydrophobic face of the α-helix provides hydrophobic membrane interaction. The large chemical shift changes shown by Trp¹¹ and N-terminal amino acid residues in SDS relative to DPC indicate that this region may be involved in membrane phospholipid recognition. ¹H-NMR assignments, CD spectra, one-dimensional ¹H-NMR spectra, chemical-shift analysis and nuclear Overhauser effect information are reported in Supplementary Publication SUP 50184 (11 pages), which has been deposited at the British Library Document Supply Centre, Boston Spa, Wetherby, West Yorkshire LS23 7BQ, U.K., from whom copies can be obtained according to the terms indicated in Biochem. J. (1997) 321, 8.

INTRODUCTION

Membrane-binding proteins, including blood-coagulation factors, interact with lipids via hydrophobic and electrostatic forces [1]. Relatively few non-integral membrane proteins have been studied structurally, and little is known about the details of the molecular interface between protein and lipid. Known structures include nisin and substance P, which maintain their respective conformations regardless of the micelle type [2,3], and β-amyloid protein fragment, whose α-helical conformation is stabilized by SDS micelles compared with dodecylphosphocholine (DPC) micelles [4]. We have previously determined structures for membrane-binding peptides [5–10] and our goal is to understand lipid–protein interactions at the atomic level and their functional significance for blood-coagulation proteins.

Factor VIII, a plasma protein of *M*_r 280 000, is a cofactor in the factor Xase enzyme complex which is essential for blood coagulation [11]. Activated factor VIII binds factor IXa and enhances its catalytic activity for factor X cleavage in the presence of phospholipids [12,13]. On maximally stimulated platelets factor VIII binds to exposed phosphatidylserine on the platelet membrane [5]. This interaction is characterized by high affinity (*K*_D ≈ 4 nM) and sufficient specificity so that no plasma protein efficiently competes for binding [14]. Precedents from other membrane-binding peptides and proteins, which lack the

specificity of factor VIII, indicate that exposure of the hydrophobic face of an amphipathic helix is a prominent mechanism for interaction with a membrane. The dependence of factor VIII on phosphoserine-containing lipids points to an ionic component to membrane binding that is not understood at the molecular level.

The membrane-binding domain of factor VIII, consisting of residues 2303–2323, fVIII_{2303–23}, competes with factor VIII for binding sites on synthetic phosphatidylserine-containing membranes and stimulated platelets [7,15,16]. The interaction of the peptide with phospholipid membranes has a *K*_D of 3 μM, which represents a free energy of 7.5 kcal/mol (1 cal ≡ 4.184 J), ~70% of the value for full-length factor VIII, and indicates a physiologically relevant conformation [5,7,14]. In the presence of DPC, as with SDS, the NMR resonance line-widths are narrow and lead to favourable spectral resolution; this is attributable to the increased motion common to some surface-associated membrane-binding peptides [17]. In SDS, fVIII_{2303–23} is amphipathic, comprising an extended N-terminal region and a C-terminal α-helix [7].

We asked if this peptide, which requires lipids to become structured, undergoes a lipid-specific conformational change. If so, what portion of the peptide is sensitive to the lipid environment? To answer these questions, we determined the structure of the peptide in DPC, which, like SDS, contains a C₁₂

Abbreviations used: fVIII_{2303–23}, membrane-binding peptide from factor VIII, 1-palmitoyl-2-stearoyl-(doxyl)-sn-glycero-3-phosphatidylcholine; DPC, dodecylphosphocholine; doxylPC, palmitoyl(doxyl)-stearoylphosphatidylcholine; NOE, nuclear Overhauser effect; RMS, root-mean-square.

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Atomic co-ordinates have been deposited with the Protein Data Bank, Brookhaven National Laboratories: file name 1CFG for the peptide in SDS and file name 1FAC for the peptide in dodecylphosphocholine.

acyl chain, but with a different head-group (phosphocholine versus sulphate) that may elicit structural changes in the peptide. We also examined the observed micelle-dependent changes in the fluorescence and CD spectra of the peptide in detail by comparing the structure of fVIII₂₃₀₃₋₂₃ in DPC micelles with the refined structure in SDS micelles.

MATERIALS AND METHODS

fVIII₂₃₀₃₋₂₃, TRYLRHPQSWVHQIALRMEV, was synthesized and purified as described previously [7]. Perdeuterated DPC (DPC-*d*₃₈) and acetic acid (acetate-*d*₄) were purchased from Cambridge Isotope Laboratories (Andover, MA, U.S.A.). Palmitoyl(doxyl)-stearoylphosphatidylcholine (doxylIPC) lipids containing the nitroxide label on either C-5 or C-12 were purchased from Avanti Polar Lipids, Alabaster, AL, U.S.A.

Fluorescence and CD spectroscopies

Fluorescence emission spectra were obtained using a Spex Fluorolog II spectrofluorimeter for samples in a 1 cm path-length quartz cuvette by exciting at 280 nm. Excitation and emission slits were set at 1 mm. Samples containing 5 μ M fVIII₂₃₀₃₋₂₃ in 50 mM NaCl and 10 mM sodium phosphate at pH 5.5 were evaluated at ambient temperature. Samples were allowed to equilibrate after the addition of phospholipid or detergent. For red-edge excitation spectroscopy excitation was at 275–310 nm; emission spectra were collected at 0.5 nm increments and were smoothed three times with an 11 data-point window.

CD studies were carried out with 24 μ M peptide as described [7].

NMR spectroscopy

NMR experiments were run on a Bruker AMX500 spectrometer. The water resonance was suppressed using pre-saturation or binomial suppression [18]. The lyophilized fVIII₂₃₀₃₋₂₃ was dissolved in 50 mM acetate-*d*₄ buffer containing 90 or 180 mM DPC-*d*₃₈ at pH 4.0 or 5.5 in 90 % H₂O/10 % ²H₂O or in 100 % ²H₂O. [The nuclear Overhauser effect spectroscopy (NOESY) spectra at the different pH values are nearly identical.] The absence of concentration-dependent changes in the sample, such as aggregation, was determined by comparing line-widths in the NMR data collected at 0.5 and 2.6 mM peptide concentrations. The peptide was stable in lipid micelle solution for several months.

pH titrations were performed with 0.5 mM peptide or L-histidine in a buffer containing 90 mM DPC-*d*₃₈ or SDS-*d*₂₅, 50 mM acetate-*d*₄ and 50 mM sodium phosphate. p*K*_a values were obtained from the H ϵ 1 chemical-shift changes by a curve-fitting routine (Microcal Software Inc., Amherst, MA, U.S.A.) using the following equation:

$$\delta_{\text{obs}} = \delta_a + (\delta_{\text{aH}} - \delta_a) \{ [\text{H}]^n / ([\text{H}]^n + K_a^n) \}$$

where δ_{obs} is the observed chemical shift at a given pH and δ_a and δ_{aH} are the calculated values for the unprotonated form and the protonated forms; [H] is the concentration of protons, *n* is the number of protons taken up or released, and *K*_a is the proton association constant.

Two-dimensional double-quantum-filtered correlation spectroscopy [19], total correlation spectroscopy [20,21] and NOESY [22] spectra were acquired, processed, assigned and analysed as described [7,23,24]. The NOESY spectra were recorded with mixing times of 50, 75, 100 and 150 ms, 96 summed scans, 2K real t₂ points, 512 t₁ increments, a spectral width of 7352.9 Hz and a relaxation delay of 1.3 s between scans. Data were processed

using either the Bruker software or Felix program (Biosym, Inc., San Diego, CA, U.S.A.). NOE intensities of cross-peaks involving H α resonances close to the water resonance were extracted from a 1-1 experiment or from data collected in ²H₂O. NOE cross-peaks were calibrated [7] and inter-proton distances were derived using a distance extrapolation procedure that takes into account spin-diffusion effects [25]. Lower bounds on the distance restraints were obtained for the short (1.8–2.8 Å), medium (2.8–4 Å), and long (> 4 Å) distances by subtracting 10 %, 15 % and 20 % respectively. Upper bounds were obtained by adding 15 %, 20 % and 25 % to the short, medium and long distances respectively. The torsion angles were obtained from one-dimensional slices of resolution-enhanced (processed with 20° shifted sine bells) NOESY [7,23,26]. χ -1 torsion angles were determined from ³*J*_{H α -H β} coupling constants and the intensities of NOE cross-peaks between α and amide protons to the β protons [27].

Structure calculation

The structure of the peptide in DPC was calculated using 443 inter-proton distance restraints and 28 torsion angle measurements and distance geometry/simulated annealing protocols [7]. Distance violations (> 0.3 Å) were compiled from ten initial structures. The upper and/or lower bounds were expanded to reflect the uncertainty in the corresponding restraints obtained from overlapping peaks. Pro-chiral assignments for methylene protons that could not be assigned unambiguously based on intra-residue distance information alone were determined by comparing the quality and final energies of the structures with each of the two possible chiralities. In the final round of calculations, all 40 of the structures converged. Six structures showed local structural anomalies in the backbone between residues Ile⁶ and Arg¹⁸, which were discarded. Using a similar protocol, the structure of the peptide in SDS micelles was subjected to a minor refinement using the NMR data collected and described previously [7], except with a correction to the covalent geometry of the tyrosine ring and the reassessment of the upper bounds on a few NOE restraints. Twenty structures were calculated, all of which converged.

Micelle-association of the peptide

The positioning of the peptide in DPC micelles was probed using doxylIPC containing doxyl groups at C-5 (5-doxylIPC) or C-12 (12-doxylIPC) atoms of the stearoyl side-chain. The peptide concentration was 1 mM. Although one-dimensional spectra gave patterns showing loss of intensity for specific protons, two-dimensional NOESY spectra were used to reduce spectral overlap. The contribution from each of the two protons was calculated as the square root of the cross-peak intensity. The loss of cross-peak intensity in the presence of either doxyl compound was normalized relative to the full intensity observed in the absence of spin label. An average was taken for all protons of each residue or as an average over the side-chain protons.

RESULTS

Fluorescence and CD of fVIII₂₃₀₃₋₂₃

Compared with fVIII₂₃₀₃₋₂₃ in buffer, the wavelength of maximum fluorescence emission for Trp¹¹ is blue-shifted by about 20 nm in the presence of DPC micelles and the fluorescence intensity is doubled (Figure 1). In SDS, the intensity is similar to that in buffer, but ~ 25 nm blue shift is observed. The blue shift seen for detergent-solubilized peptide indicates protection of the tryptophan from solvent molecules. The differences in fluorescence

Figure 1 (A) Fluorescence emission spectra of fVIII₂₃₀₃₋₂₃ and (B) red-edge excitation shift analysis

(A) The fluorescence emission spectrum of 5 μ M peptide in phosphate buffer (50 mM NaCl/10 mM sodium phosphate, pH 5.5) shows an emission maximum at about 355 nm. Upon addition of 5 mM SDS or DPC, the emission maximum is blue-shifted to 330 nm or 335 nm respectively. In DPC micelles, there is a 2-fold increase in the fluorescence intensity. The excitation wavelength was 280 nm. (B) A change in the maximum fluorescence emission wavelength was monitored as a function of the excitation wavelength for fVIII₂₃₀₃₋₂₃. The peptide in SDS micelles (■) shows a 23 nm red shift in the maximum fluorescence emission wavelength when the excitation wavelength is increased from 270 nm to 310 nm. A shift of 8 nm is observed for the peptide in buffer alone (○), whereas the peptide in DPC micelles (●) shows no red-edge excitation shift.

spectra between SDS and DPC imply that either the peptide conformation near the tryptophan is not the same, or that Trp¹¹ interacts directly and in a different manner with the two detergent micelles. Upon increasing the excitation wavelength from 270 nm to 310 nm, the peptide's maximum wavelength of fluorescence emission was found to red shift by 8 nm in buffer (Figure 1), 23 nm in SDS and 0 nm in DPC. The 8 nm red shift in buffer is consistent with that predicted for a solvent-exposed tryptophan [28]. The large 23 nm red shift in the emission maximum in SDS indicates that the indole ring exists in a polar environment of restricted mobility. In contrast, the invariance of the emission maximum for the peptide in DPC indicates that the indole ring is located in an environment that is relatively non-polar or highly mobile.

The far-UV CD spectra of fVIII₂₃₀₃₋₂₃ were obtained in phosphate buffer, SDS micelles and DPC micelles. [¹H NMR resonance assignments, CD spectra, one-dimensional ¹H-NMR spectra, chemical-shift analysis and NOE information are reported in Supplementary Publication SUP 50184 (11 pages), which has been deposited at the British Library Document Supply Centre, Boston Spa, Wetherby, West Yorkshire LS23 7BQ, U.K, from whom copies can be obtained according to the terms indicated in Biochem. J. (1997) 321, 8.] The spectrum in buffer has a minimum at 200 nm; spectral shape is consistent with a largely random-coil conformation. In the presence of

Figure 2 Fingerprint region of a representative two-dimensional NOE spectrum of fVIII₂₃₀₃₋₂₃

Intra-residue HN-H α connectivities are labelled with the single letter code followed by the residue number for all residues. Some of the cross-peaks of Pro⁸ H α with the side-chain of Trp¹¹ are also indicated. Experimental conditions: 2.7 mM peptide, 180 mM DPC, 50 mM sodium acetate buffer, pH 4.0, 308.3 K, and 75 ms NOE mixing time.

DPC or SDS [7] the spectra are similar and contain minima at 208 and 222 nm, characteristic of an α -helical conformation. This indicates a similar overall conformation in both micelles.

Structure determination of fVIII₂₃₀₃₋₂₃

Dispersed resonances in the ¹H-NMR spectrum of the peptide in DPC indicates the presence of ordered secondary structure (Figure 2 and Supplementary Publication SUP 50184). Several resonances are at frequencies different from those in SDS micelles. For example, the Trp¹¹ ϵ 1 proton shifts from 10.05 p.p.m. in SDS to 10.61 p.p.m. in DPC. Peptide secondary structure was determined from chemical shift index analysis, NOE connectivities and ³J_{HN-H α} coupling constants. The amide and α proton chemical shifts of fVIII₂₃₀₃₋₂₃ in DPC micelles differ from random-coil chemical shifts (Supplementary Publication SUP 50184) and indicate that residues Pro⁸-Met¹⁹ of fVIII₂₃₀₃₋₂₃ are helical [29]. Nearly continuous $\alpha\beta_{(i,i+3)}$ NOE cross-peaks are observed for these residues. The ³J_{HN-H α} coupling constant of residues Tyr³, Arg⁵, His⁷ and Ser¹⁰ are between 6 and 8 Hz and that of Ile⁶ is 9 Hz; it was less than 6 Hz for all other residues. Thus, residues Trp¹¹-Met¹⁹ are in a well-defined α -helix and the N-terminal segment of the peptide, comprising residues Thr¹-Ile⁶, is largely unstructured or consists of a turn. These results are consistent with a peptide backbone structure that is preserved on associating with SDS and DPC micelles and with lipid-dependent changes in the environment of selected amino acid side-chains.

Refinement based on the NMR data for the peptide in SDS resulted in a modest improvement in definition of the structure. Root-mean-square (RMS) deviation for superimposition of the peptide backbone over Tyr³-Arg¹⁸ is 0.4 ± 0.1 Å (Figure 3). In

Figure 3 Overlay of peptide structures for Ile⁶–Met¹⁹ of fVIII_{2303–23} in stereoview

Shown are 20 structures in SDS (**A**) and 20 structures in DPC micelles (**B**). The backbone torsion angles, ϕ and ψ , of residues Ile⁶–Arg¹⁸ are well-defined for the peptide in both micelles. The backbone atoms of Ile⁶–Arg¹⁸, calculated using distance geometry and simulated annealing, have been superimposed on the corresponding atoms of the lowest energy structure. The illustration includes Met¹⁹ because it is part of the peptide's hydrophobic face. Side-chains of positively charged residues are in black, uncharged polar residues are in dark grey, and hydrophobic residues and Met¹⁹ are in lighter grey. The mean RMS deviation for superimposed atoms is 0.7 ± 0.2 Å for structures in DPC micelles and 0.4 ± 0.1 for the refined structures in SDS micelles. (**C**) Structure of fVIII_{2303–23} in SDS (black) and in DPC (grey) micelles. Backbone atoms of amino acids Ile⁶–Arg¹⁸ of the lowest energy structures calculated for the peptide in SDS and DPC micelles are superimposed (RMS deviation = 1.5 Å) and are shown along with the Trp¹¹ side-chains to emphasize repositioning of the side-chain.

our previous description of the structure the RMS deviation was 0.6 ± 0.2 Å for Leu⁴–Met¹⁹ [7]. The structures have the same conformation as described before and contain a C-terminal helix and an N-terminal extended region.

In DPC, the peptide structure is nearly as well defined, and,

interestingly, contains a similar C-terminal helix and an N-terminal extended region. The backbone ϕ and ψ dihedral angles are uniformly well-defined from Ile⁶ to Arg¹⁸, with an angular order parameter [30] of $S \geq 0.9$. The mean RMS deviation for pair-wise superimposition of residues 6–18 is 0.7 ± 0.2 Å for the

backbone atoms and 1.7 ± 0.3 Å for all other atoms. The set of 20 refined structural models in SDS (Figure 3A) and 20 structures randomly chosen from the set of 34 in DPC (Figure 3B) illustrate the structural similarities and differences in the different lipid environments. Analysis of the dihedral angle information of residues 6–18 for the lowest energy structure of fVIII_{2303–23} in SDS and in DPC, using the Procheck program [31], showed that 90% are in the most favoured regions and the remaining 10% are in additional allowed regions, reflecting a well-defined structure. The peptide backbone at the extreme N- and C-termini is less defined, and the NOE and line-width data indicate that these residues are mobile and unstructured. Within the well-ordered segment comprising residues Ile⁶–Arg¹⁸, the side-chain positions of residues Gln⁹, His¹³, Gln¹⁴ and Arg¹⁸ are not well-defined. Interestingly, the backbone and side-chain dihedral angles of Tyr³ are well-defined in DPC micelles, even though the residues flanking it are unordered. The side-chains of several residues show NOE cross-peaks to one another and form a hydrophobic patch on one face of the peptide (Figure 3). The opposing face of the peptide is hydrophilic.

We determined the χ -1 and χ -2 dihedral angles of W11 carefully so as to understand the lipid-induced differences in the fluorescence spectrum of fVIII_{2303–23}. χ -1 and χ -2 dihedral angles ($S > 0.9$) show that the Trp¹¹ side-chain is in the *trans*, +90 conformation in DPC micelles [32], whereas it is *gauche*, -0 in SDS micelles. The χ -2 dihedral angle is defined through several short-range NOEs to the backbone and side-chain protons of its own and neighbouring residues. The Trp¹¹ amide and α protons show strong NOEs to the δ 1 proton and weak NOEs on the ϵ 3 proton. The β protons have strong NOEs on the δ 1 proton and medium-strength NOEs on the ϵ 3 proton. Cross-peaks are also observed between protons of the indole ring and Pro⁸ α , Ser¹⁰ β , Ile¹⁵ methyl groups and Val¹² methyl groups; the conformation of this region was checked by comparing the observed NOE intensities with those back-calculated from the structure [25]. We observed that the intensities of several of these cross-peaks are altered for the peptide in SDS micelles. For example, cross-peaks between Trp¹¹ ϵ 1 and Pro⁸ α , Trp¹¹ ϵ 3 and Val¹² α , Trp¹¹ ϵ 3 and Ile¹⁵ β , and Trp¹¹ ζ 3 and Val¹² α are weaker in SDS, and those between Trp¹¹ η 2 and Ile⁶ methyl groups and Trp¹¹ δ 1 and Ser¹⁰ β s are stronger [7]. Most of the NOE intensities between Pro⁸ and Trp¹¹ are weaker in DPC micelles than in SDS micelles, indicating the increased inter-atomic distance between these two residues (Figure 3). For example, the α -proton of Pro⁸ is spatially close to the δ 1, ϵ 1 and ϵ 3 protons of the Trp¹¹ side-chain and exhibits a large change in chemical shift (-0.3 p.p.m. in DPC relative to SDS). Consequently, although the overall backbone structure of fVIII_{2303–23} is similar in DPC and in SDS micelles, the reoriented Trp¹¹ side-chain tips towards the micelle interior in DPC rather than pointing toward the surface as in SDS micelles (Figure 3C).

The largest ¹H chemical-shift changes in DPC relative to those in SDS micelles are in the extended region of the peptide, and the conformation of this region is altered. The biggest change is 0.6 p.p.m. for the amide proton of Gln⁹. The chemical shift for H ϵ of Arg² and Arg⁵, H α of Tyr³, and H β of Leu⁴ are all changed by more than 0.15 p.p.m.. Thus, the extended region of the peptide is involved in sensing the change in lipid type between SDS and DPC.

Positioning of fVIII_{2303–23} in detergent micelles

The pK_a values measured for His⁷ and His¹³ imidazole rings in DPC micelles (6.09 ± 0.04 , 6.27 ± 0.02) are similar to the pK_a for the free L-histidine in SDS (6.24 ± 0.02) or DPC (6.33 ± 0.03) micelles and for free histidine (6.00) in aqueous solution [33].

However, the His⁷ and His¹³ side-chains exhibit a higher pK_a value (7.35 ± 0.05 , 7.28 ± 0.05) in SDS micelles, indicating that the imidazole ring experiences an acidic or electron-rich environment and places the solvent-exposed histidines in the acidic interfacial region of the SDS micelles [34,35]. The unaltered pK_a values of the two histidines in DPC indicate that the imidazole rings are near the overall neutral charge of the DPC headgroup. The pK_a data are consistent with the three-dimensional structure and with our model for association of the peptide's hydrophobic face with the micelle surface, which places the histidines on the hydrophilic face of the peptide.

The addition of 24 μ M CoCl₂ quenched 27% of the fluorescence of 5 μ M fVIII_{2303–23} at pH 5.5 in the absence of micelles (results not shown). In the presence of submicellar detergent concentration (50 μ M), fluorescence quenching by CoCl₂ was comparable with that in the absence of micelles and indicated that either the peptide did not associate with the lipids or the associating lipids do not protect the tryptophan side-chain from bulk solvent. In the presence of either DPC or SDS micelles, fluorescence quenching was less than 10%, showing that Trp¹¹ is protected from the aqueous phase due to burial of the side-chain. The blue-shifting of the maximum fluorescence emission on association with micelles and the effects of CoCl₂ on the fluorescence intensity indicate burial of the tryptophan.

Information from line-broadening in ¹H-NMR resonances caused by lipid-soluble spin-labelled compounds (doxylPC) was used to determine the position of the peptide relative to the micelle surface. We used two doxylPC lipids with the doxyl group located at either the C-5 or the C-12 positions of the stearyl side-chain. The 5-nitroxide label is located close to the phosphate group at the micelle surface and the 12-doxyl group is near the centre of the micelle [36,37]. Thus, peptide protons at the surface of the micelle will not be affected strongly by either the label at C-5 or at C-12. Protons located at a depth of 3 Å and at 9 Å from the surface (C-2 carbon is considered to be at the surface) will be most affected by the 5-doxyl and 12-doxyl groups respectively [38]. The least affected residues of the peptide are Ser¹⁰, His¹³, Ala¹⁶, Leu¹⁷, Arg¹⁸, Glu²⁰ and Val²¹, localizing these residues to the hydrophilic face of the peptide (Supplementary Publication SUP 50184). 5-DoxylPC caused the largest changes in Pro⁸, and to a lesser degree, in Tyr³ and Ile⁶. Therefore, these residues interact with the lipid acyl chain near the interfacial region of the micelle. The residues affected most by 12-doxylPC are Leu⁴ and Met¹⁹, followed by Pro⁸, Tyr³, Ile⁶, Trp¹¹, Ile¹⁵, and, surprisingly, the side-chain of the polar residue Gln¹⁴, and are most buried. Thus, side-chains of Tyr³, Leu⁴, Ile⁶, Pro⁸, Trp¹¹, Gln¹⁴ and Met¹⁹ interact with the acyl chains of the lipids and penetrate deeper into the micelle.

As indicated by the doxyl data, the α -helix of fVIII_{2303–23} lies on the surface of DPC micelles and the side-chains of the hydrophobic residues reach into the acyl region of the micelle, which is in agreement with the topographical representation that we proposed previously [7]. Anomalies such as lipid-tail re-orientation and micelle shape modification can result from peptide–lipid interactions [39] and lead to inaccurate depth measurements. However, glucagon, a 29-residue peptide that associates with micelle surface, does not distort the micelle shape [40,41], suggesting that the micelle shape remains unmodified upon surface association of our 21-residue peptide. Further, the pK_a values of His⁷ and His¹³ in DPC micelles, equivalent to histidine dissolved in buffer, show that the hydrophilic face of the peptide containing the histidine residues is exposed to bulk solvent. The direction and magnitude of the pK_a shift for both histidines in SDS, relative to DPC micelles (see the Results section), locates the histidines in the acidic shell at the micelle

surface [34,35]. Thus the only orientation which satisfies the positional constraints of the hydrophobic residues and of the histidine residues is one in which the molecular long axis of fVIII₂₃₀₃₋₂₃ is parallel to the micelle surface, with the α -helix backbone at the interfacial region of the micelle and the long axis of its α -helix parallel to the micelle surface, and with side-chains of selected residues in the extended N-terminal portion, as well as in the α -helix, penetrating the micelle.

DISCUSSION

We have determined and analysed the structure of fVIII₂₃₀₃₋₂₃ in DPC micelles to gain insight into the specific effects of phosphorylcholine, the dominant ionic moiety in the outer leaflet of plasma membranes, on the membrane-binding region of factor VIII. The conformation is compared with the refined structure in SDS micelles. The conformation of the C-terminal portion is similar in both micelles, except that in SDS the first turn of the α -helix (residues Pro⁸-Ser¹⁰) is more compact [7]. Thus, the amphipathic α -helix, a common motif in membrane-binding peptides and proteins, forms in response to the hydrophobic-hydrophilic interface of both micelle types and does not reflect specific chemical interactions with the lipid.

In contrast, the conformation of Trp¹¹ (and N-terminal residues Arg²-Ser¹⁰) changed dramatically. In DPC micelles, increased fluorescence of Trp¹¹ announces an altered chemical environment for the indole ring. The structural data show that this moiety resides in the micelle interior. The identity of the peptide side-chain moieties that may act as fluorescence acceptors or quenchers do not vary between these two model membrane environments and provide no explanation for the 2-fold difference in fluorescence intensity. The 2-fold increase in the fluorescence-intensity in DPC micelles relative to SDS can be explained by decreased quenching, perhaps from deeper burial of the tryptophan side-chain [42].

Exciting the polar fluorophores, in polar, viscous solvents at the red edge of its excitation spectrum, produces a red shift in the maximum of the emission spectrum (red-edge excitation shift) [28,43,44]. A remarkable feature of the fluorescence of fVIII₂₃₀₃₋₂₃ in DPC micelles is the absence of red-edge excitation shift which is observed in SDS (Figure 1). This result is expected for a specific interaction of the Trp¹¹ indole ring with DPC in which there is no significant subpopulation with fluorophore-solvent dipole-dipole interaction that has a reduced energy difference between the ground and excited states; it implies a non-viscous, a non-polar or a non-rigid environment. That the surface or interior of DPC micelles is less viscous than aqueous buffer may be dismissed. More likely, the indole ring resides sufficiently deep in the fluid-like interior of DPC micelles so that there are no polar interactions, an idea that is consistent with the observed increase in fluorescence intensity and the positioning of the indole ring determined using doxylIPC.

fVIII₂₃₀₃₋₂₃ is recognized by anti-factor VIII antibodies that block the membrane-binding function of factor VIII, indicating that the 21 amino acid residue peptide assumes the native membrane-binding structure of the full-length protein [7]. Studies with inhibitory anti-factor VIII antibodies suggest that the extended region of the peptide (Thr¹-Ser¹⁰) contains a phospholipid-recognition motif. The monoclonal antibody ESH8, with an epitope corresponding to residues 2248-2307, does not block binding of factor VIII to phosphatidylserine-containing membranes [7]. A human alloantibody called TK, which recognizes an epitope corresponding to residues 2249-2312, blocked binding of factor VIII to phosphatidylserine in microtitre wells [45]. These data suggest that residues 2307-2312

(Arg⁵-Ser¹⁰ in the peptide) are important for recognition of phosphatidylserine, and possibly phosphatidylcholine. We note this region of the peptide had the largest conformational and NMR chemical-shift difference in DPC compared with SDS.

This report describes the structure of fVIII₂₃₀₃₋₂₃ comprising a C-terminal membrane binding motif and an N-terminal lipid-sensing motif. Whereas the C-terminal amphipathic α -helix provides a membrane-binding function without specificity for lipid type, the N-terminal extended region, encompassing residues Arg²-Trp¹¹, mediates specific polar interactions. A distinct conformational change is seen in response to lipid-type at Trp¹¹, which is located at the junction of the extended region and the α -helix, and the results are consistent with this residue being constrained due to an interaction that is phosphocholine-dependent. The results are consistent with recognition by the parent molecule, factor VIII, of a membrane-binding site consisting of phosphocholine as a moiety of phosphatidylcholine or sphingomyelin. Additional studies are under way to determine the precise interactions that occur between the N-terminal region of fVIII₂₃₀₃₋₂₃ and the lipid head-groups at the membrane surface.

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