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Membrane interaction of 'peptide P' derived from the repeating motif of properdin

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A 24 amino acid residue peptide corresponding to the central part of the 'thrombospondin-repeat' motif of the human serum protein properdin was synthesized. The peptide, termed 'peptide P', contains three tryptophans near the N-terminus and an arginine cluster close to the C-terminus. Its sequence closely matches a consensus sequence which has been claimed to characterize a sulfatide binding motif. Membrane binding of peptide P was analyzed using changes in its tryptophan emission upon adding small unilamellar vesicles. The peptide bound to the membranes in a way suggesting simple water/membrane partitioning. Analysis of electrostatic effects at different ionic strengths indicated small electrostatic contributions upon interaction with zwitterionic lipid, despite the large charge number ($z = +4$) of the peptide. Membrane affinity was increased by one order of magnitude if the bilayers contained 20% of negatively charged lipid. No difference could be detected whether the charged lipid was sulfatide or phosphatidylglycerol. Strong and rapid vesicle aggregation was evident as the peptide associated with the negatively charged vesicles. In addition, a fluorescent energy transfer assay with vesicles and internal total reflection fluorescence microscopy on supported bilayers were used to study membrane interaction of whole human properdin. No sulfatide specificity could be detected.

Introduction

Properdin is a plasma glycoprotein that plays a stabilizing role in the alternative pathway of the complement system. Sequence studies showed that the molecule is made up from six structurally similar motifs of 58 amino acid residues each [1]. Since these motifs are homologous to three structural domains originally found in thrombospondin [2], they were termed 'thrombospondin repeats' (TSRs). These TSR domains are also found in all the components that constitute the 'membrane attack complex' (MAC) of the complement system, namely C6, C7, C8α, C8β and C9 [3]. Part of the TSR motif is present in the circumsporozoite proteins (CS) of several species of malaria parasites and in a protein, derived from the merozoite stage of *Plasmodium falciparum*, termed thrombospondin-related anonymous protein (TRAP) [4].

The functional role of this motif remains at present unknown; in view of its presence in the complement components that form the MAC, and in the CS proteins of malaria parasites, membrane interaction has been proposed as one possibility [1]. In fact, properdin has recently been shown to bind to membranes containing sulfatides [5].

In order to investigate those membrane interactions further, we have used a synthetic peptide, 'peptide P', 24 amino acid residues long, which represents the central part of the 'thrombospondin-repeat' motif. Its sequence, which is shown below, is derived from the second homologous repeat of human properdin [6]:

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1                   5                  10
Trp Ser Gly Trp Gly Pro Trp Glu Pro Cys Ser Val

          15                  20
Thr Cys Ser Lys Gly Thr Arg Thr Arg Arg Arg Ala
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Abbreviations: DPhs-PC, 1-(3-(diphenylhexatrienyl)propanoyl)-2-palmitoyl-*sn*-glycero-3-phosphocholine; POPC, 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine; POPG, 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphoglycerol; sulfatide, Gal(3-SO_4)1-1-ceramide; DTT, dithiothreitol; FITC, fluoresceine isothiocynate; Trp, tryptophan; HPLC, high-performance liquid chromatography; TLC, thin-layer chromatography.

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The sequence is characterised by three equally spaced tryptophans in the N-terminal half and an arginine cluster near the C-terminus. The sequence from residue 10–23 represents the consensus sulfatide binding motif described by Holt et al. [5], apart from a serine in position 15 instead of a glycine. At neutral pH, the peptide is expected to bear a net charge of $z = +4$ electronic units.

Changes in the fluorescence properties of the three tryptophans have been used to monitor association of this peptide to small unilamellar vesicles made out of synthetic lipids. Special attention was given to the binding to negatively charged lipids and the question whether there is a special effect of sulfatides. The same question was also addressed in additional experiments with whole human properdin.

Peptide P is of further interest as a member of a group of charged amphiphilic peptides. There is growing evidence for an apparent suppression of electrostatic effects upon membrane binding of such peptides (see e.g. the discussion in Ref. 7), especially if the charges are borne by arginine residues. The arginine cluster in peptide P would make it a valuable model for investigating such phenomena.

Materials and Methods

Synthesis of 'peptide P'

Peptide P was synthesized using an Applied Biosystems model 420A automated peptide synthesizer by the solid phase method. The synthesis was performed on a *p*-methylbenzhydrylamine resin using *t*-butyloxylcarbonyl-protected amino acids (Bachem, Switzerland). The couplings were performed in dimethylformamide/dichloromethane using dicyclohexylcarbodiimide as the coupling agent, followed by neutralization with *N,N*-diisopropylethylamine and 40% trifluoroacetic acid for deprotection. The coupling efficiency was followed after each cycle by the Kaiser ninhydrin test [8]. After completion of the synthesis, the F-moc protected side chains were deprotected using 10% pyridine in dimethylformamide. The final products were cleaved from the resin by highly anhydrous HF in the presence of thioanisole at a ratio of HF/anisole/peptide resin of 10:1:1 for 1 h at 4°C [9], and after washing the resin with cold diethyl ether, the peptide was extracted with 10% acetic acid. The crude peptide was purified by gel filtration on a Sephadex G-15 column equilibrated with 2% acetic acid followed by reverse-phase HPLC on a C_{18} (Vydac) column, using a 60 min gradient of 0 to 80% acetonitrile containing 0.1% trifluoroacetic acid. The purity and composition of the peptide were confirmed by TLC and amino acid analysis using an Applied Biosystems 420A gas phase derivatizer with an on line 130A analyzer.

Purification of human properdin

A Sepharose anti-properdin monoclonal antibody column was prepared by coupling a mouse monoclonal antibody (supplied by Dr. C. Koch, Statens Seruminstitut, Denmark) to CNBr-activated Sepharose 4B. The column (1.5 cm × 7 cm), containing 3.5 mg of antibody per ml of Sepharose 4B, was equilibrated in phosphate-buffered saline (pH 7.4) (PBS). Serum (2 liter) that had been extensively dialysed against PBS was preabsorbed with Sepharose 4B and then applied to the monoclonal antibody column which was then extensively washed with PBS. Any bound material was eluted first with PBS made 3 M with respect to NaCl and then with 0.2 M glycine-0.5 M NaCl (pH 2.5). The pH 2.5 eluate was immediately neutralized by the addition of 1 M NaOH. This fraction was reapplied to the affinity column and eluted, using the same conditions as before. The overall yield was 7 mg of properdin from 1 liter of serum.

Properdin was fluorescently labeled with FITC according to standard procedures, with a final degree of labelling of 0.6 fluorophores per protein molecule.

Methods

Peptide concentrations were determined from the optical absorbance at 280 nm, assuming an absorption coefficient of $3 \times 5700\ M^{-1}\ cm^{-1}$ [10]. Properdin concentrations were determined using an absorption coefficient of $120000\ M^{-1}\ cm^{-1}$ (on a monomer basis), in agreement with Perkins et al. [11].

Lipids were purchased from Avanti, Pelham, AL (POPC, POPG) and from Supelco, Bellefonte, PA (sulfatide). Small unilamellar vesicles were prepared by sonifying the hydrated lipid with a titanium tip at 10°C, until the solutions became clear, followed by centrifugation in an Eppendorf ultracentrifuge for 10 min in order to remove titanium debris. Lipid concentrations in the vesicle solutions were checked by phosphate analysis [12]. Standard buffer was 10 mM Tris-HCl, (pH 7.1), with 0.5 mM EDTA and 0.1 M NaCl added. Properdin was dialyzed against this buffer before use in the vesicle binding experiments. In some cases, the NaCl concentration was changed as indicated explicitly in the text. Further addition of DTT to the solutions containing peptide P was without any effect on the results.

Fluorescence was recorded in a thermostatted quartz cuvette in a Schoeffel RS1000 fluorometer. Excitation wavelength was 280 nm. The bandwidth was 2 nm in the excitation and 4 nm in the emission path. The (very small) signal contribution of pure vesicles in buffer was subtracted. Inner filter effects were corrected using the known absorbance of the peptide solution in the center of the cuvette.

Supported lipid bilayers were prepared according to published procedures [13] via consecutive apposition of

two monolayers (surface pressure 32 mN/m) onto a quartz slide. The first monolayer was always POPC, the second, outer layer was either POPC or sulfatide. Binding to the supported bilayers was measured using total internal reflection fluorescence microscopy [13]. Total internal reflection illumination is only capable of exciting fluorophores in the vicinity of the quartz-buffer interface, with a characteristic penetration depth of about 100 nm. The measured fluorescence intensity therefore is mainly due to molecules bound to the membrane. These experiments were done in 200 mM glycine buffer (pH 8), 2 mM $CaCl_2$.

Results

A. Peptide P

1. Tryptophan spectra

The tryptophan emission spectrum of peptide P is centered around about 358 nm, thus indicating that the Trp residues are largely exposed to water (Fig. 1).

The fluorescence intensity (corrected for inner filter effects) at the peak wavelength remained proportional to the aqueous peptide concentration up to the highest concentration checked (15 μM). Thus, there was no indication for any aggregation of the peptide in this concentration range.

When small unilamellar vesicles were added, the emission peak shifted to lower wavelength, together with an increase in intensity (Fig. 1). In the presence of excess lipid, where the peptide is thought to be nearly fully associated with the vesicles (cf. evaluation below), the emission peak is found at 348 nm (with an estimated uncertainty of at least 1 nm mainly due to spectral distortion by light scattering and to incomplete binding; the two effects tend to compensate each other, however). The spectral intensity thereby increases by about 50% (uncharged lipid) to 60% (charged lipid). The shift of the emission peak wavelength was similar under all conditions studied, but the amount of lipid needed to produce a given shift (i.e., the strength of interaction) depended strongly on the charge of the membrane and the salt conditions (Fig. 1).

Thus, none of the Trp residues seems to dip deeply into the hydrophobic core, since there was no component discernible whose emission peak had shifted well below 340 nm.

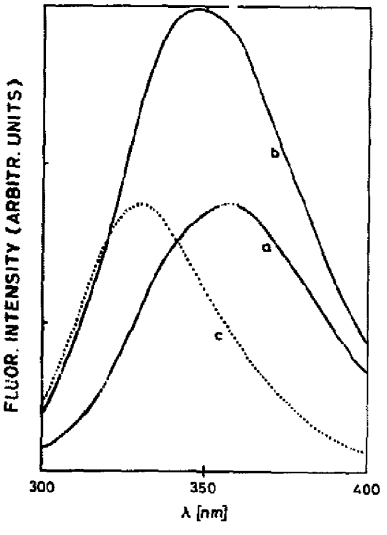

Fig. 1. (Uncorrected) tryptophan emission spectra of 5 μM peptide P in buffer (a) and after addition of 360 μM POPC/POPG, 80:20 mol/mol (b), and for 190 nM properdin (c, scale enlarged by about a factor of 7 with respect to a).

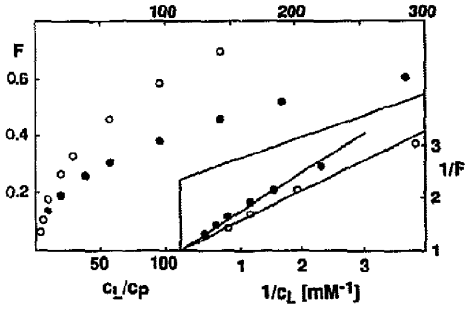

Fig. 2. Relative fluorescence intensity increase, F, at 340 nm upon titrating peptide P (9.2 μM (○) and 4.6 μM (●) with POPC vesicles. NaCl concentration: 0.1 M. (Inset) Inverse plot, $1/F$ versus reciprocal of lipid concentration, for extrapolation of limiting value of F.

2. Binding to lipid vesicles

In the present context, the term 'binding' is used in a broad sense. It does not necessarily mean binding to well defined saturable sites, but could also apply to some sort of partitioning into the membrane phase. Binding (partitioning) parameters could be obtained from the change of the fluorescence signal, ΔS, relative to the signal in the absence of lipid, S_0, as a function of the amount of lipid material added to the peptide solutions. These changes were evaluated at a fixed wavelength of 340 nm.

The relative signal increase, $F = \Delta S/S_0$, is plotted in Fig. 2 as a function of the lipid to peptide ratio, c_L/c_P, for titrations of the zwitterionic lipid, POPC, to peptide solutions of various concentrations. The asymptotic value of the signal, F_{inf}, corresponding to fully bound peptide, was determined by extrapolation from an inverse plot of $1/F$ versus $1/c_L$, as shown in the inset to Fig. 2. With the value of $F_{inf} = 1$ obtained in this way, the amount of bound peptide per lipid, r, and of free aqueous peptide, c, could be calculated

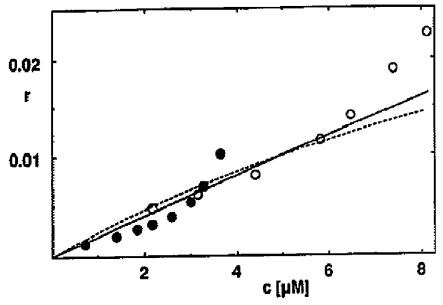

Fig. 3. Bound peptide per lipid, r, versus free aqueous peptide, c, calculated from data in Fig. 2. Symbols are as in Fig. 2. Straight line: ideal partition equilibrium; dashed line would correspond to electrostatic effects for a peptide carrying an effective charge of 1.2, see text for details.

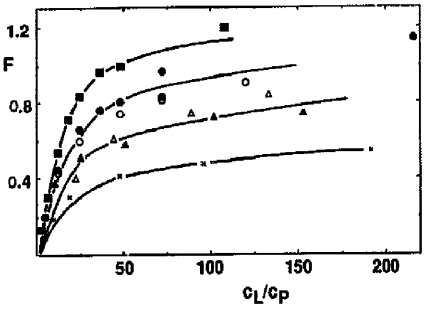

Fig. 4. Titration of various concentrations of peptide P (4.6 μM, squares; 2.3 μM, circles; 1.1 μM, triangles; 0.57 μM, crosses) with vesicles made from POPC/POPG 80:20 mol/mol (full symbols) and POPC/sulfatide 80:20 mol/mol (open symbols). Lines are drawn by hand to guide the eye.

(see Ref. 14 for details). We refer to an r versus c plot as an 'isotherm' (Fig. 3).

Titrations were also performed including 20% of negatively charged lipid, either POPG or sulfatide, in the bilayers. In these cases, a very rapid (seconds) and strong visible increase in turbidity was noted after adding the vesicles to the peptide solutions. Obviously, the peptide strongly aggregates or fuses small unilamellar vesicles. Speed and extent of this effect were in fact stronger than with the well-known fusogen, melittin.

In order to rule out experimental artefacts arising from turbidity, we did the following control experiment: a half-micro cuvette was placed once with the short lightpath in the emission, and once in the excitation beam. Light scattering being strongly wavelength dependent, differential effects should result for the two orientations, if the measured signal were seriously affected by the turbidity change. However, the relative signal changes remained identical.

In order to limit the turbidity increase, single points in the titrations were repeated using freshly prepared solutions. In addition, turbidity problems were found to be absent when using very low peptide concentrations (< 1 μM).

Fig. 4 gives the relative signal increase as a function of the lipid-to-peptide ratio, for various peptide concentrations. The isotherm (Fig. 5) was calculated in the same way as described above, with F_{inf} determined as 1.2 (using the criterion of overlapping isotherms [14] in addition to inverse-plot extrapolations). The experiment at the lowest concentration suffices to determine the initial slope of the isotherm, equal to an 'apparent partition coefficient' in the presence of negatively charged lipid. Extension of the isotherm to higher free concentrations is subject to some uncertainty due to the turbidity problems, but in view of the above-mentioned controls should nevertheless represent a valuable estimate.

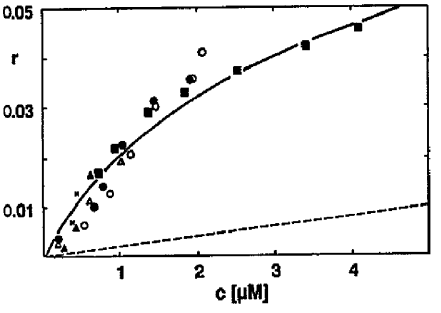

Fig. 5. Bound peptide per lipid versus free peptide, calculated from data in Fig. 4. Symbols are the same as in Fig. 4. Line is fit with parameter values given in the text. Dashed line is for pure POPC (equal to full line in Fig. 2).

Replacing the POPG in the bilayers by sulfatide, the appearance of the samples as well as the quantitative signal changes were precisely equal to those obtained with POPG (Figs. 4 and 5).

Titrations with the zwitterionic lipid, POPC, were also repeated at different salt concentrations (1 M NaCl and no NaCl, respectively, added to the buffer instead of 100 mM). With the high NaCl concentrations, slight turbidity effects also appeared at high lipid

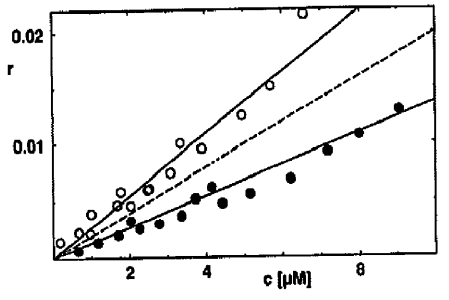

Fig. 6. Bound peptide per lipid versus free peptide with POPC at 1 M NaCl (open circles) and without NaCl (full circles). Dashed line indicates situation at 0.1 M NaCl, taken over from Fig. 3.

concentrations. Isotherms at different salt concentrations (all evaluated using $F_{inf} = 1$) are compared to each other in Fig. 6.

B. Properdin

Some experiments were also performed with whole human properdin. The fluorescence spectrum of this protein in the Trp emission region showed a single peak, centered at 330 nm (Fig. 1). The tryptophans in properdin thus appear to be shielded from the solvent by folding and, probably, by aggregation (the protein is known to form head-to-tail aggregates in solution, cf. Ref. 1).

Experimentally, we could not find changes in the emission intensity neither, which could have been used for binding studies similar to those described for peptid P above. We therefore turned to two other techniques in order to test membrane interactions of properdin in general and the role of sulfatides in particular.

1. Fluorescence energy transfer

DPH-PC was incorporated to 5% into bilayers formed from POPC and 20% charged lipid (either POPG or sulfatide) and vesicles formed by short (15 min) sonication. Samples were illuminated at 280 nm and the DPH emission recorded at 435 nm.

Stepwise addition of properdin lead to a corresponding increase of the fluorescence signal, apparently due to resonance energy transfer from the properdin trp residues to the DPH acceptor (Fig. 7). No turbidity was detected (nor was it expected to occur in view of the extremely low concentrations used: protein, 40–400 nM, lipid, 8–9 μM). Signal increase was larger upon adding $CaCl_2$ to a final concentration of 2 mM (Fig. 7).

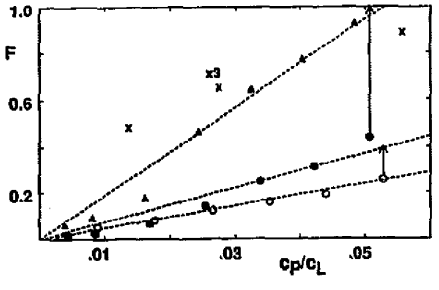

Fig. 7. Relative fluorescence intensity increase, F, due to energy transfer from properdin tryptophans to DPH-PC (5 mol%) in vesicles made out of POPC/POPG (80:20, full symbols) and POPC/sulfatide (80:20, open symbols). Salt conditions: 0.1 M NaCl, 0.5 mM EDTA, without (circles) and with (triangles) 2 mM $CaCl_2$ added. Arrows indicate effect of addition of 2 mM $CaCl_2$ at the end of the titration. Crosses: Peptide P addition to POPC/POPG 80:20 vesicles containing 5% DPH-PC, at a 3-fold reduced scale (no $CaCl_2$).

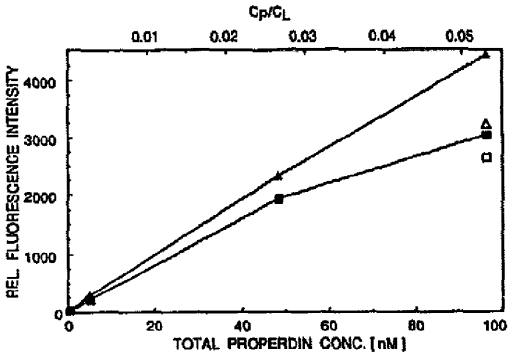

Fig. 8. Binding of FITC-properdin to supported planar bilayers containing POPC (squares) or sulfatide (triangles) in the outer leaflet, measured by total internal reflection fluorescence microscopy. At the end point of the titrations, done in the presence of 2 mM $CaCl_2$, EDTA was added to a final con-centration of 3.3 mM (open symbols). Lipid concentration was 1.9 μM.

Repeating the experiments with POPC/sulfatide/DPH-PC instead of POPC/POPG/DPH-PC, produced no significant difference (Fig. 7).

The same fluorescent energy transfer assay was also applied to the peptide P instead of properdin, under identical conditions of salt and lipid concentration. The peptide yielded stronger signal increase, as shown in Fig. 7 on a 3-fold reduced scale.

2. Properdin binding to supported bilayers

Total internal reflection fluorescence microscopy was used to determine the binding of FITC-labelled properdin to supported lipid bilayers containing POPC in the inner and either POPC or sulfatide in the outer leaflet. No saturation could be seen in the concentration range investigated (up to 100 nM, $c_P/c_L = 0.55$). The difference between the isotherms obtained with POPC and sulfatide bilayers, respectively, was very small (30% less fluorescence intensity with the uncharged membrane at the highest properdin concentration). These experiments were performed in the presence of 2 mM $CaCl_2$ (Fig. 8). Addition of 3.3 mM EDTA had no significant effect on properdin binding to uncharged POPC membranes, but decreased binding to the negatively charged sulfatide membrane by about 30%.

Discussion

Peptide P, a 24 residue peptide from the 'thrombospondin repeat' (TSR) motif of properdin, has been shown to bind to phospholipid bilayers. Binding isotherms are essentially straight lines (Figs. 3, 6) without any indication of saturable sites, so 'binding' is most readily understood in terms of a water/membrane partition equilibrium.

The changes in the tryptophan fluorescence allow quantitation of partition parameters: the partition coefficient, Γ, as defined by an ideal partition equilibrium

$$r = \Gamma_c \tag{1}$$

equals 2000 M^{-1} for the neutral (zwitterionic) lipid, POPC. This figure corresponds to a moderate membrane affinity, of the order of magnitude found for the well-studied model peptide alamethicin (1000 M^{-1}, [14]) or the signal peptide of cytochrome-*c* oxidase subunit IV (2300 M^{-1}, [15]), but an order of magnitude smaller than that of the strongly surface seeking peptide melittin (30000 M^{-1}, [16]) or the signal peptides of bacterial phosphotransferases (34000 M^{-1}, [15]).

Strictly speaking, the isotherms found in the present work have a slight tendency of bending upwards, though this may not be significant in view of experimental uncertainties. (At all peptide concentrations investigated, the very first points in the titrations tended to be too high; a similar effect has previously been noted with the model peptides melittin and alamethicin (Stankowski, S., unpublished data) and may be due to disruption of some vesicles at low lipid-to peptide ratios; the corresponding points were discarded from our isotherms). However, there is clearly no flattening (downward bending) of the curves, as one might have expected from the large number of charges (net charge $z = 4$) borne by the peptide at neutral pH. As more and more peptide accumulates at the surface of the vesicles, charge repulsion should tend to discourage further binding, as is well known e.g. from melittin [16], a peptide which carries a similar basic charge cluster near its C-terminus. In the case of melittin, four of its six charges present at neutral pH are contributed by the N-terminus and three lysines, the remaining two are arginines. From titrating some of these charges it has recently been concluded that the two arginines do not contribute very much to membrane charging [7]. One may then speculate that there is a similar effect in peptide P, where four arginines are present.

On the other hand, an apparent increase in bilayer affinity is obvious if negatively charged lipids are included. The initial slope of the isotherm in Fig. 5 corresponds to an apparent partition coefficient $\Gamma_{app} = 20000\ M^{-1}$, about 10-times larger than for the neutral lipid. This increase in bilayer affinity may be explained by direct binding of basic residues to negative lipid or, more simply, by electrostatic attraction of the peptide by the negative surface potential of the charged bilayer. The surface potential, ψ, of a 20% charged bilayer under our salt conditions is readily calculated from Gouy-Chapman theory to be -52 mV. Γ_{app} is then expected to be related to the intrinsic partition coefficient, Γ, by a Boltzmann factor (cf. Ref. 16 for details):

$$\Gamma_{app} = \Gamma/\alpha = \Gamma \cdot \exp(z_{eff} \cdot \psi \cdot e_0 / kT) \tag{2}$$

where e_0 is the electronic charge and kT is thermal energy. α represents an activity coefficient in the bilayer phase. The experimental value of the exponential term being 10, the effective surface charge of the peptide, z_{eff}, is obtained as 1.15.

We recall that this value is based on the initial slope of the isotherm alone. A fit of the whole curve can be done, using the formalism given in Ref. 16. For the 20% negative bilayers we obtain in this way a partition coefficient $\Gamma = 2700\ M^{-1}$ and $z_{eff} = 1.2$. This is close to the direct estimate given above. The fitting line is plotted in Fig. 5. The same parameters also give a reasonable fit to the POPC isotherm (dashed curve in Fig. 3), but of course cannot reproduce the slight bending upwards at high *c* values. Ideal partitioning (straight full line in Fig. 3) without electrostatic contributions therefore still appears to better describe the data in the case of the uncharged bilayers.

Clearly, a z_{eff} value between 0 (straight line) and 1.2 (curbed fit line) is very low for a peptide of net charge equal to 4. However, similar effective charge reduction has been found with other peptides [16–18], especially if the charges in question were arginines [7,19]. Up to date, it remains obscure whether this remarkable effect stems from a still undetermined molecular mechanism or a deficiency in treating multivalently charged molecules by the Gouy-Chapman theory [20].

The salt dependence of the partition equilibrium should give additional information about electrostatic interactions. We therefore repeated the experiments with POPC vesicles in the presence of high (1 M) NaCl concentration and without any NaCl added to 10 mM of buffer. The bilayer affinity increased at high salt concentration ($\Gamma = 2750\ M^{-1}$) and decreased at low salt ($\Gamma = 1350\ M^{-1}$). These changes were different from those expected from Gouy-Chapman theory. The latter would have predicted primarily an effect on the activity coefficient, i.e., on the degree of bending of the isotherms. Instead, the isotherms remain linear at all salt concentrations, but the partition coefficient changes.

Of course, the partition coefficient relates not only to the affinity for the bilayer phase, but also to that for the water phase; the aqueous activity coefficient also changes by varying the salt concentration. In this way, the experimental values of the partition coefficients can easily be rationalized. That the overall shape of the isotherm remains essentially linear strongly suggests that the linearity at 100 mM NaCl is not due to a fortuitous balance between electrostatic repulsion and attractive (e.g., aggregation in the membrane phase, cf.

Ref. 14) interactions, but to the virtual absence of surface charging in the case of the neutral membranes.

From a physiological point of view it is interesting to note from our study that peptide P, the central part of the homologous 'thrombospondin repeat' motif in properdin, terminal complement proteins and thrombospondin, has a natural affinity for lipid membranes. This affinity is increased if the membrane contains negative lipids (by one order of magnitude with 20% acidic lipid). However, we could not find any specific requirement for sulfatides which had previously been claimed to be a natural receptor for properdin (and other proteins harboring a similar 'sulfatide-binding motif', cf. Ref. 5). In contrast, other acidic lipids, such as phosphatidylglycerol, did as well. The effect was explainable by simple electrostatic charge interactions without any need of other, more specific interactions.

From two types of other experiments, the same conclusion was found to hold for the whole protein of human properdin. The latter was shown to bind with comparable affinity to both POPC/POPG and POPC/sulfatide containing lipid bilayers, using an energy transfer assay with lipid vesicles. Similarly, very small differences between POPC and sulfatide were found in total internal reflection fluorometry on supported lipid bilayers. Calcium ions increased the binding to negatively charged membranes roughly 2-fold, but not to zwitterionic lipids like POPC.

At present we do not know the reason for the discrepancy between the sulfatide specificity as reported by Holt et al. [5] using a lipid plating assay, and the lack of sulfatide specificity in our systems, i.e., lipid vesicles and supported bilayers. Further study will be needed to fully clarify this issue.

Our results obtained with the peptide P strongly suggest implication of the N-terminal tryptophans in the interaction with membranes. Electrostatic interactions are important in promoting affinity of the peptide to charged membranes, but are much weaker than expected from the presence of four arginine residues. This concerns not only binding interactions in the strict sense, but even Gouy-Chapman effects due to the simple presence of charges near a bilayer. Such evidence suggests that the arginine containing C-terminal part of the peptide stays relatively far from the membrane surface.

The tryptophans of peptide P are found to play an active role in membrane interactions. Since the corresponding residues in properdin appear to be mainly buried in the interior of the protein aggregate, dissociation of the aggregate and/or conformational changes of the protein might be required to expose these residues for membrane interactions.

Acknowledgements

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