FEBS Letters 481 (2000) 96–100 FEBS 24090

Membrane topology of VacA cytotoxin from *H. pylori*

Xiao-Ming Wang[a], Ruddy Wattiez[b], Cristina Paggliacia[c], John L. Telford[c], Jean-Marie Ruysschaert[a], Véronique Cabiaux[a,*]

[a]*Structure et Fonction des Membranes Biologiques, CP 206/2, Université Libre de Bruxelles, Boulevard du Triomphe, B-1050 Brussels, Belgium*
[b]*Service de Chimie Biologique, Université de Mons-Hainaut, Avenue du Champ de Mars 6, 7000 Mons, Belgium*
[c]*IRIS, Chiron SpA, Via Fiorentina, 1, 53100 Siena, Italy*

Received 25 July 2000; accepted 11 August 2000

Edited by Maurice Montal

Abstract The interaction of VacA with membranes involves: (i) a low pH activation that induces VacA monomerization in solution, (ii) binding of the monomers to the membrane, (iii) oligomerization and (iv) channel formation. To better understand the structure–activity relationship of VacA, we determined its topology in a lipid membrane by a combination of proteolytic, structural and fluorescence techniques. Residues 40–66, 111–169, 205–266, 548–574 and 723–767 were protected from proteolysis because of their interaction with the membrane. This last peptide was shown to most probably adopt a surface orientation. Both α-helices and β-sheets were found in the structure of the protected peptides.

Key words: VacA cytotoxin; Membrane; *Helicobacter pylori*

1. Introduction

The toxin VacA produced by pathogenic *Helicobacter pylori* strains is a major virulence factor in human gastroduodenal diseases [1–5]. The protein is synthesized as a 140 kDa precursor from which a 40 kDa C-terminal domain necessary for extracellular secretion is removed on the bacterial surface [6]. The mature protein (95 kDa) is found both at the bacterial cell surface and in the extracellular medium. This 95 kDa protein purified from the supernatants of *H. pylori* frequently contains two fragments of 37 kDa (p37 residues N-ter-319) and 58 kDa (p58 residues 320-C-ter) [6]. The full length protein that is released by the bacteria is a low activity complex formed by up to 12 monomers (Fig. 1.1) [6,7]. This complex can be activated by a low pH treatment which most probably results in oligomer disassembly (Fig. 1.2) [7,8]. The treatment of cells with activated VacA leads to the formation in the cell cytoplasm of large V-ATPase-, rab 7- and Igp-positive vacuoles originating from late endosomal and lysosomal compartments [9] and to a vacuolation-independent permeability increase of polarized epithelial monolayers [10,11]. Recently, it has been demonstrated that low pH-activated VacA was able to induce anion-selective channels in planar lipid bilayers and HeLa cells plasma membrane [12,13]. The increased permeability of the plasma membrane of gastric epithelial cells by VacA might help *H. pylori* colonization of the stomach by allowing the efflux of potential metabolic substrates such as pyruvate and HCO_3^-. Whether vacuolation is a late consequence of the channel formation or the consequence of a cytoplasmic interaction between VacA, or a VacA domain, and a still non-identified target remains to be determined.

The pH dependence of VacA-induced vacuolation reveals that maximum activity is reached slightly below pH 5.0 and remains stable down to pH 2 [14]. Using sucrose gradient centrifugation it was demonstrated that, at pH 5.0, the protein undergoes a transition from an oligomeric to a monomeric structure [14]. This transition was characterized by exposure at the surface of the protein of hydrophobic sites, as evidenced by binding of ANS, a fluorescent hydrophobic probe [14]. Binding of VacA to lipid vesicles was also maximum at pH 5.0 and was characterized by the formation of hexameric pores in the lipid membrane [15]. Furthermore, activation at a mild acidic pH leads to a deep insertion of VacA in the lipid membrane as demonstrated by labeling by hydrophobic photoactivatable lipid probes [16]. The current model that is proposed for the interaction of VacA with the membrane involves (i) a low pH activation that induces a VacA monomerization in solution (Fig. 1.2), (ii) binding of the monomers to the membrane (Fig. 1.3), (iii) oligomerization (Fig. 1.4) and (iv) channel formation (Fig. 1.5). To better understand the structure–activity relationship of VacA, we have probed the topology of this protein inserted at pH 5.0 in liposomes by proteolysis and fluorescence studies. The secondary structures of the membrane peptides were determined by Fourier transform infrared spectroscopy (FTIR).

2. Materials and methods

2.1. Materials

Proteinase K (PK) and asolectin (mixed soybean phospholipids) were obtained from Sigma (St. Louis, MO, USA). Phenylmethylsulfonyl fluoride (PMSF) was from Serva. Polyvinylidene difluoride (PVDF; problott) membranes were obtained from Applied Biosystems, and acrylamide was from Bio-Rad. All other reagents were of the highest purity available.

2.2. Protein purification

VacA from *H. pylori* strain CCUG 17847 was purified from the broth culture supernatants as described in [17]. For all experiments, the protein was purified by gel filtration on a Superose 6 column in 2 mM 4-(2-hydroxyethyl)-1-piperazine ethanesulfonic acid (HEPES), 2 mM NaCl, pH 7.2 and stored at 4°C.

2.3. Liposome preparation

Asolectin was purified according to the method of [18] and kept as

*Corresponding author. Fax: (32)-2-650 5382.
E-mail: vcabiaux@ulb.ac.be

Abbreviations: FTIR, Fourier transform infrared spectroscopy; HEPES, 4-(2-hydroxyethyl)-1-piperazine ethanesulfonic acid; LUV, large unilamellar vesicle; PMSF, phenylmethylsulfonyl fluoride; PVDF, polyvinylidene difluoride

PII: S0014-5793(00)01978-5

a stock solution in chloroform (100 mg/ml). A film of lipids was formed on a glass tube under a nitrogen flux. This film was rehydrated in a 20 mM HEPES, 150 mM NaCl, pH 7.2, buffer. Large unilamellar vesicles (LUV) were formed by an extrusion procedure (pores: 0.1 μm diameter) at room temperature according to [19]. The concentration of lipids was determined by measuring the lipid phosphorus content [20].

2.4. Preparation of VacA proteoliposomes

300 μg of VacA was mixed with LUV (VacA/LUV, 1/10, w/w in a final volume of 350 μl) in a 20 mM HEPES, 150 mM NaCl, buffer at pH 7.2, and incubated for 10 min at 37°C. The pH was then lowered to pH 5.0 by addition of a predetermined volume of 1 M sodium acetate (NaAc), pH 5.0. Under these conditions, 100% of VacA was inserted into the liposomes [21]. After 10 min of incubation at 37°C, the sample was run on a 30–2% sucrose gradient as described in [22] and the protein and lipid distributions along the gradient were determined, respectively, by measuring Trp fluorescence (λ_{ex} = 280 nm and λ_{em} = 340 nm) and by choline dosage (Test combination phospholipids, Boehringer Mannheim Biochemia). Fractions which contained both lipids and proteins were pooled, centrifuged and washed twice to remove the sucrose with (i) a 2 mM HEPES, 2 mM NaCl, pH 5.0, buffer (FTIR experiments) or (ii) a 20 mM NaAc, 150 mM NaCl, pH 5.0, buffer (PK experiments) or (iii) a 20 mM Tris, 150 mM NaCl, 2 mM EDTA, pH 7.0 buffer (cysteine binding to *N*-[6-(7-amino-4-methylcoumarin-3-acetamido)hexyl]-3′-(2′-pyridyldithio)propionamide (AMCA-HPDP)).

2.5. Proteolysis of VacA proteoliposomes by PK

To determine the proteolysis conditions, a freshly prepared stock solution of PK (5 mg/ml) was added to the VacA proteoliposomes prepared as previously described (VacA 0.6 mg/ml, PK/VacA, 1/1, w/w). $CaCl_2$ was added at a final concentration of 2 mM. The incubation was carried out at 37°C for time periods of 1, 5, 10, 24 and 48 h. The digestion was stopped by addition of 2 mM PMSF. The sample was then mixed with an equal volume of 80% sucrose, and overlaid with a 30–2% linear sucrose gradient. After an overnight centrifugation at 125 000 × *g* at 4°C (Beckman L7 ultracentrifuge, SW60 rotor), the proteoliposomes were recovered and centrifuged twice in a 2 mM HEPES, 2 mM NaCl, buffer at pH 5.0 to remove the sucrose. The digested samples were delipidated [23] and run either on a 5–20% acrylamide gradient gel electrophoresis (sample buffer: 62 mM Tris–HCl, pH 6.8, buffer, 2% sodium dodecyl sulfate (SDS), 10% glycerol (w/v) and 0.01% bromophenol blue) [24] or a Tris–Tricine SDS–polyacrylamide gel electrophoresis (PAGE) (16.5% combined acrylamide and bisacrylamide (T), 6% of which is crosslinker (bisacrylamide, C) [25]. To prepare the samples for N-terminal sequencing and structural analysis, the VacA proteoliposomes were digested at a PK/VacA ratio of 1/1 (w:w) at 37°C for 48 h. The peptides N-terminal amino acid sequence was determined as described in [23].

2.6. Cysteine labeling

Binding of the fluorescent probe AMCA-HPDP (1 mg/ml in dimethyl sulfoxide) was performed at pH 7.0 on VacA in solution and VacA reconstituted in lipid vesicles, before and after proteolysis and in the absence or presence of a reducing agent according to [23]. The reduction step was carried out for 2 h in the presence of a 100-fold excess of dithiothreitol (DTT) in a 20 mM Tris, 150 mM NaCl, 2 mM EDTA, pH 7.0 buffer. The reducing agent was removed by centrifugation of the samples through a G50 mini-column. The protein was labeled with a 20-fold molar excess of AMCA-HPDP for 1 h at room temperature and when proteoliposomes were involved, 0.15% Triton X-100 was added to the sample. In all cases, the unreacted AMCA-HPDP and detergent were eliminated by gel filtration on a G50 mini-column equilibrated with 0.1% SDS in water. The fluorescence associated with VacA was measured with an SLM 8000 spectrofluorimeter (λ_{ex} = 345 nm; λ_{em} = 450 nm), and the protein concentration was determined by Trp fluorescence using a standard curve obtained with VacA in 0.1% SDS.

2.7. IR spectroscopy

Attenuated total reflection infrared spectra (resolution of 4 cm^{-1}) were obtained with a Perkin-Elmer 1720X FTIR spectrophotometer as previously described [26]. Measurements and sample deuteration were carried out as described in [27,28]: hydrogen/deuterium exchange allows differentiation of the α-helix from the random structure, whose absorbance bands shift from about 1655 to about 1642 cm^{-1}. The determination of the secondary structure of proteins was carried out by analysis of the deuterated amide I region as described previously [27]. The frequency limits for the different structures were as follows: 1662–1645 cm^{-1}, α-helix; 1689–1682 cm^{-1} and 1637–1613 cm^{-1}, β-sheet; 1645–1637 cm^{-1}, random coil; and 1682–1662 cm^{-1}, β-turns. The control spectra of the 2 mM HEPES buffer, pH 7.2, and asolectin vesicles at both pH 5.0 showed no absorbance between 1700 and 1600 cm^{-1} (data not shown).

3. Results

Proteoliposomes containing VacA inserted in the membrane were prepared by incubating the protein with LUVs of asolectin at low pH. The lipid-bound protein was separated from the non-associated protein by centrifugation on a sucrose gradient. To determine the regions of VacA protected from a protease because of their association to the membrane, VacA proteoliposomes were treated with a non-specific protease, PK. Digestion was performed at 37°C at a PK/VacA ratio of 1/1 (w/w) from 0 to 48 h, and the samples were analyzed by electrophoresis on a 5–20% gradient SDS–PAGE gel. After 48 h, no full length protein was detectable on the gel (Fig. 2). Instead, a large band, most probably containing several proteolysed peptides was observed at the bottom of the gel. A sample obtained after 48 h of digestion was therefore run on a Tris–Tricine electrophoresis gel (16.5% T, 6% C) which should allow the resolution of peptides from 2 to 20 kDa [25]. Even under these conditions, the bands were not fully resolved and the molecular weights of the peptides ranged from 13 to about 3 kDa (data not shown). These

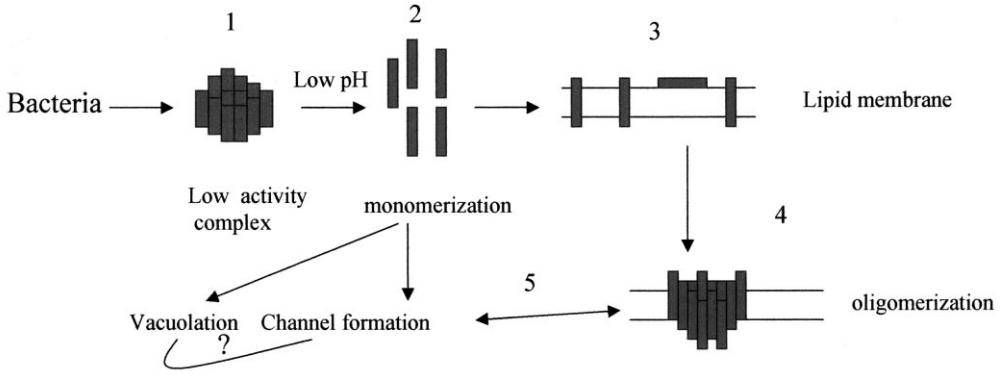

Fig. 1. Schematic representation of VacA mechanism of interaction with a lipid membrane.

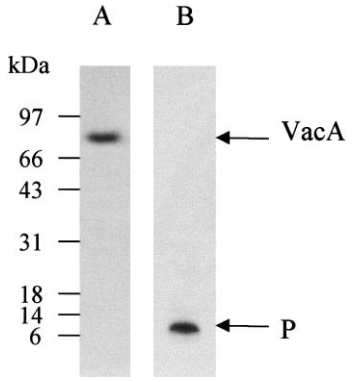

Fig. 2. Proteolysis of VacA proteoliposomes. Silver staining of a 5–20% gradient SDS–PAGE gel. A: 5 μg of VacA reconstituted in LUV as described in Section 2. B: VacA proteoliposomes (300 μg VacA) treated for 48 h by PK as described in Section 2. P indicates the presence of several unresolved peptides.

bands were transferred onto a PVDF membrane which was sliced into six zones in the region containing the peptides. The N-terminal amino acid sequence analysis was then applied to these six samples (Table 1). Each sample contained more than one peptide. In total, five N-terminal moieties were identified: N39 (or K40), T111, L205, S548 and N723. The approximate C-terminal boundaries of the protected regions were evaluated from the smallest obtained peptides, according to their apparent molecular weights as determined from the gel (about 3 kDa for peptides N39, K40 and S548, about 5 kDa for peptide N723 and about 6 kDa for the other ones). The higher molecular weight peptides most probably correspond to partial digestion. The minimal protected sequences are reported on the VacA sequence in Fig. 3. Three nearby protected regions (residues 40–66, 111–169 and 205–266) are located at the

Table 1
N-terminal sequences of the protected peptides

Molecular weight (kDa)	Band	N-terminal sequence
12.8	A1	^{40}KTPDKPDKVWRIQAGKGFNEF
	A2	^{39}NKTPDKPDKVWRIQAGKGFNEF
	A3	^{205}LYDGATLNLASSS
	A4	^{548}SRINTVRLETGTRSLFSG
	A5	^{723}NVNLEEQFKERLALYN
10.4	B1	^{40}KTPDKPDKVWRIQAGKGFNEF
	B2	^{39}NKTPDKPDKVWRIQAGKGFNEF
	B3	^{723}NVNLEEQFKERLALYN
	B4	^{548}SRINTVRLETGTRSLFSG
8.4	C1	^{723}NVNLEEQFKERLALYN
	C2	^{40}KTPDKPDKVWRIQAGKGFNEF
	C3	^{39}NKTPDKPDKVWRIQAGKGFNEF
	C4	^{548}SRINTVRLETGTRSLFSG
	C5	^{111}TLSGLRNFTGGDLDVNM
	C6	^{205}LYDGATLNLASSS
6.4	D1	^{723}NVNLEEQFKERLALYN
	D2	^{40}KTPDKPDKVWRIQAGKGFNEF
	D3	^{39}NKTPDKPDKVWRIQAGKGFNEF
	D4	^{548}SRINTVRLETGTRSLFSG
	D5	^{111}TLSGLRNFTGGDLDVNM
	D6	^{205}LYDGATLNLASSS
About 5	E1	^{723}NVNLEEQFKERLALYN
	E2	^{40}KTPDKPDKVWRIQAGKGFNEF
	E3	^{39}NKTPDKPDKVWRIQAGKGFNEF
About 3	F1	^{548}SRINTVRLETGTRSLFSG
	F2	^{40}KTPDKPDKVWRIQAGKGFNEF
	F3	^{39}NKTPDKPDKVWRIQAGKGFNEF

```
-33  MEIQQTHRKI NRPLVSLALV GALVSITPQQ SHAAFFTTVI IPAIVGGIAT GTAVGTVSGL
 28  LSWGLKQAEE ANKTPDKPDK VWRIQAGKGF NEFPNKEYDL YRSLLSSKID GGWDWGNAAR
 88  HYWVKGGQQN KLEVDMKDAV GTYTLSGLRN FTGGDLDVNM QKATLRLGQF NGNSFTSYKD
148  SADRTTRVDF NAKNISIDNF VEINNRVGSG AGRKASSTVL TLQASEGITS DKNAEISLYD
208  GATLNLASSS VKLMGNVWMG RLQYVGAYLA PSYSTINTSK VTGEVNFNHL TVGDKNAAQA
268  GIIANKKTNI GTLDLWQSAG LNIIAPPEGG YKDKPNNTPS QJGAKNDKNE SAKNDKQESS
328  QNNSNTQVIN PPNSAQKTEV QPTQVIDGPF AGGKDTVVNI NRINTNADGT IRVGGFKASL
388  TTNAAHLHIG KGGVNLSNQA SGRSLIVENL TGNITVDGPL RVNNQVGGYA LAGSSANFEF
448  KAGTDTKNGT ATFNNDISLG RFVNLKVDAH TANFKGIDTG NGGFNTLDFS GVTDKVNINK
508  LITASTNVAV KNFNINELIV KTNGISVGEY THFSEDIGSQ SRINTVRLET GTRSLFSGGV
568  KFKGGEKLVI DEFYYSPWNY FDARNIKNVE ITNKLAFGPQ GSPWGTSKLM FNNLTLGQNA
628  VMDYSQFSNL TIQGDFINNQ GTINYLVRGG KVATLSVGNA AAMMFNNDID SATGFYKPLI
688  KINSAQDLIK NTEHVLLKAK IIGYGNVSTG TNGISNVNLE EQFKERLALY NNNNRMDTCV
748  VRNTDDIKAC GMAIGDQSMV NNPDNYKYLI GKAWKNIGIS KTANGSKISV YYLGNSTPTE
808  NGGNTTNLPT NTTSNARS
```

Fig. 3. Localization in the VacA sequence of the protected peptides. The arrow indicates the N-terminal moiety determined by N-terminal sequencing. The C-terminal residues are defined on the basis of the apparent molecular weights of the peptides, as determined by electrophoresis on a Tris–Tricine gel (16.5% T, 6% C).

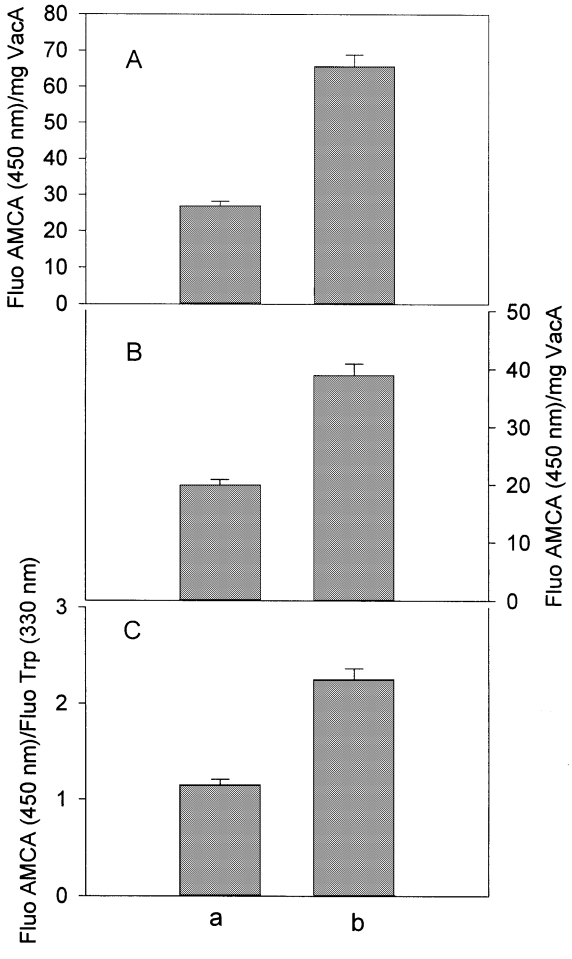

Fig. 4. Binding of AMCA-HPDP to soluble (A) or membrane-associated VacA without (B) or with (C) proteolysis. A and B: the concentration of VacA was determined as described in Section 2. (a) Absence of DTT; (b) reduction with DTT. All fluorescence measurements were performed in 0.1% SDS (see Section 2). Four, three and two experiments were carried out, respectively, in A, B and C.

N-terminal moiety of the protein. They are followed by a long unprotected domain (from about residue 264 to 547). Two protected peptides are identified at the C-terminal extremity of the protein (residues 548–574 and 723–767).

The proteolytic approach permits the identification of peptides that are tightly bound to a lipid membrane but does not formally provide information about the transmembrane or surface orientation of these peptides. We took advantage of the presence of two cysteine residues in VacA (Cys746 and Cys757) to provide data on the membrane topology of the protected peptide 723–767. We investigated this topology by measuring the binding of the probe AMCA-HPDP to the protein. This fluorescent probe binds to free thiols. The amount of bound AMCA-HPDP was determined by the protein fluorescence ($\lambda_{ex} = 345$ nm; and $\lambda_{em} = 450$ nm) after removal of the unbound probe. In the absence of DTT, AMCA binds to VacA in solution at neutral pH (Fig. 4A). This binding is increased upon addition of DTT to the suspension suggesting the presence of two populations of VacA, characterized by the presence, or absence, of a disulfide bridge: according to the fluorescence data, about 40% of the VacA molecules do not contain this bridge. Addition of a denaturing concentration of guanidine hydrochloride (6 M) did not increase the signal obtained in the presence of DTT (not shown) suggesting that all Cys residues are located at the surface of the protein. VacA proteoliposomes were then prepared at pH 5.0 as previously described and washed in a Tris buffer at pH 7.0. Fig. 4B shows that the binding of AMCA to proteoliposomes in the absence or presence of DTT was qualitatively similar to that observed in solution. Similar results were obtained with the VacA proteoliposomes treated by PK (Fig. 4C). In the last two cases, the addition of Triton X-100 to disrupt the liposomes prior to AMCA binding did not increase the fluorescence obtained in the presence of DTT (data not shown). This strongly suggests that the Cys residues of lipid-bound VacA and of peptide 723–767 are fully accessible to AMCA-HPDP and that the protected peptide 723–767 adopts a surface orientation.

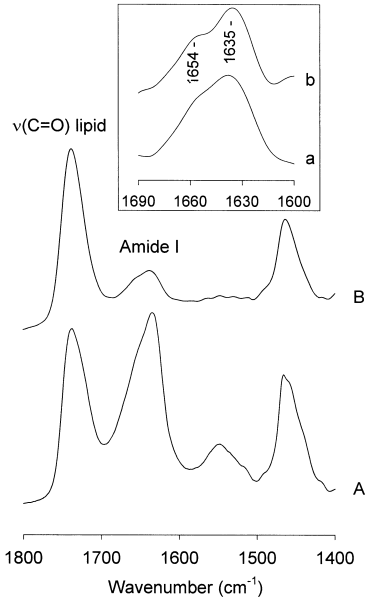

Fig. 5. Infrared spectra of deuterated VacA proteoliposomes (A) before and (B) after proteolysis carried out as described in Section 2. Inset: Amide I region corresponding to spectrum b (a) without and (b) with deconvolution (resolution factor 2).

According to the FTIR spectroscopy analysis of VacA, this protein contains $24 \pm 5\%$ and $35 \pm 5\%$ of α-helices and β-sheets, respectively [21]. The FTIR spectroscopy is based on the analysis of the vibration bands of protein and particularly the ν(C=O) of the peptidic bond (amide I band), whose frequency of absorption is strongly dependent upon the secondary structure. It was used here to determine the secondary structure of the membrane-protected peptides. Fig. 5 shows the deuterated spectra of proteoliposomes of VacA before and after digestion with PK. After digestion, the surface ratio of amide I/lipid ν(C=O) decreased, which confirms the removal of most of the VacA residues. Fig. 5, inset, gives the spectrum in the amide I region of the digested sample. It is characterized by absorption maxima at 1635 cm^{-1} (β-sheet) and 1654 cm^{-1} (α-helix). The percentages of secondary structures for intact and proteolysed lipid-bound VacA are, respectively, 24 and 30% of α-helix, 35 and 44% of β-sheets, 18 and 15% of random coils and 23 and 11% of β-turns.

4. Discussion

To provide further information on the structure–activity relationship of VacA, we aimed at determining the membrane-associated regions of the protein as well as their structure. When a non-specific protease was added to VacA proteoliposomes, a first group of protected peptides covers residues 40–66, 111–169 and 205–266 indicating that the N-terminal region of VacA has a strong tendency to interact with the membrane. This N-terminal region is responsible for several properties of VacA:

- Formation of water-soluble oligomeric structures (Fig. 1.1): short deletions between residues 28 and 294 lead to molecules unable to oligomerize in water [29].
- Channel formation: full length VacA induces channel formation but neither the N-terminal (p37) nor the C-terminal (p58) alone are responsible for this property suggesting that domains from the two subunits are essential for channel formation [10,13].
- Vacuolation: the minimal vacuolating domain has been localized between residues 1 and 422. Any N-ter, C-ter or internal deletions of this region leads to a VacA protein devoid of its vacuolating activity [29–31].

Channel formation is one of the most likely steps that would require the insertion of protein domains in the lipid membrane and it is therefore tempting to suggest that the protected peptides 40–66, 111–169 and 205–266 play a role in channel formation. A special mention should be made of residues 6–27. Based on the hydrophobicity of this region, it was suggested that it could interact with the membrane. A VacA mutant deleted of residues 6–27 induces channel formation with a conductance identical to the WT protein but at a slower rate and with a modified selectivity [29]. The lipid-binding properties as well as the ability to oligomerize into the lipid membrane (Fig. 1, steps 3 and 4) of this mutant are unaltered [29]. This indicates that residues 6–27 might not be essential for membrane insertion and regulate the channel properties by an unknown mechanism. Our proteolysis data

provide a further indication that residues 6–27 are indeed not membrane associated.

As discussed above, the N-terminal region of VacA is necessary but not sufficient to induce channel formation. Two additional protected peptides were found at the C-terminal moiety between residues 548 and 574 and residues 723 and 767. The last peptide is most probably associated to the surface of the lipid membrane, according to the accessibility of Cys 746 and 757 to the fluorescent probe AMCA-HPDP. Whether the two peptides are associated to channel formation is unclear. Binding properties have been associated to the C-terminal of VacA: antibodies reactive to amino acids 479–803 inhibit VacA binding to cells [32]. We cannot, therefore, exclude that association of the C-terminal protected peptides to the membrane could be related to cell surface binding instead of, or in addition to, channel formation.

We previously demonstrated that the secondary structure of VacA was independent of the pH and the presence of a lipid membrane. This structure contains $24 \pm 5\%$ and $35 \pm 5\%$ of α-helices and β-sheets, respectively [21]. The proportion of α-helix and β-sheets increased in the proteolysed sample suggesting that the peptides protected by the lipid membrane are highly organized and contain both types of structure. A similar observation has been made for diphtheria toxin, an A-B type toxin in which the N-terminal part of the B domain, which carries the channel-forming domain, interacts with the membrane through α-helices and the C-terminal moiety, carrying the receptor binding site via β-sheets [23,33].

In conclusion, we have localized the domains of VacA protected from an externally added protease by their interaction with a lipid membrane. Peptides from both the N- and C-terminus of VacA have been identified and both α-helix and β-sheet structures are found within these peptides. Further work will have to be carried out to understand the role of these peptides in cell binding, vacuolation and channel formation of VacA but this study provides a good basis for site-directed mutagenesis studies.

Acknowledgements: This work was supported by the EC grant Biomed2 BMH4 CT97-2410. V.C. is Senior Research Associate and R.W. is scientific researcher of the Fonds National pour la Recherche Scientifique (Belgium).

References

[1] Marshall, B.J. and Warren, J.R. (1984) Lancet 1, 1311–1315.
[2] Leunk, R.D., Johnson, P.T., David, B.C., Kraft, W.G. and Morgan, D.R. (1988) J. Med. Microbiol. 26, 93–99.
[3] Parsonnet, J., Hansen, S., Rodriguez, L., Gelb, A.B., Warnke, R.A., Jellum, E., Orentreich, N., Vogelman, J.H. and Friedman, G.D. (1994) New Engl. J. Med. 330, 1267–1271.
[4] Cover, T.L. (1996) Mol. Microbiol. 20, 241–246.
[5] Cover, T.L. and Blaser, M.J. (1992) J. Biol. Chem. 267, 10570–10575.
[6] Telford, J.L., Ghiara, P., Dell'Orco, M., Comanducci, M., Burroni, D., Bugnoli, M., Tecce, M.F., Censini, S., Covacci, A. and Xiang, Z. (1994) J. Exp. Med. 179, 1653–1658.
[7] Cover, T.L., Hanson, P.I. and Heuser, J.E. (1997) J. Cell. Biol. 138, 759–769.
[8] de Bernard, M., Papini, E., de Filippis, V., Gottardi, E., Telford, J., Manetti, R., Fontana, A., Rappuoli, R. and Montecucco, C. (1995) J. Biol. Chem. 270, 23937–23940.
[9] Papini, E., Satin, B., Bucci, C., de Bernard, M., Telford, J.L., Manetti, R., Rappuoli, R., Zerial, M. and Montecucco, C. (1997) EMBO J. 16, 15–24.
[10] Papini, E., Satin, B., Norais, N., de Bernard, M., Telford, J.L., Rappuoli, R. and Montecucco, C. (1998) J. Clin. Invest. 102, 813–820.
[11] Pelicic, V., Reyrat, J.M., Sartori, L., Pagliaccia, C., Rappuoli, R., Telford, J.L., Montecucco, C. and Papini, E. (1999) Microbiology 145, 2043–2050.
[12] Szabo, I., Brutsche, S., Tombola, F., Moschioni, M., Satin, B., Telford, J.L., Rappuoli, R., Montecucco, C., Papini, E. and Zoratti, M. (1999) EMBO J. 18, 5517–5527.
[13] Tombola, F., Carlesso, C., Szabo, I., de Bernard, M., Reyrat, J.M., Telford, J.L., Rappuoli, R., Montecucco, C., Papini, E. and Zoratti, M. (1999) Biophys. J. 76, 1401–1409.
[14] Molinari, M., Galli, C., de Bernard, M., Norais, N., Ruysschaert, J.M., Rappuoli, R. and Montecucco, C. (1998) Biochem. Biophys. Res. Commun. 248, 334–340.
[15] Czajkowsky, D.M., Iwamoto, H., Cover, T.L. and Shao, Z. (1999) Proc. Natl. Acad. Sci. USA 96, 2001–2006.
[16] Moll, G., Papini, E., Colonna, R., Burroni, D., Telford, J., Rappuoli, R. and Montecucco, C. (1995) Eur. J. Biochem. 234, 947–952.
[17] Manetti, R., Massari, P., Burroni, D., de Bernard, M., Marchini, A., Olivieri, R., Papini, E., Montecucco, C., Rappuoli, R. and Telford, J.L. (1995) Infect. Immun. 63, 4476–4480.
[18] Kagawa, Y. and Racker, E. (1971) J. Biol. Chem. 246, 5477–5487.
[19] Hope, M.J., Bally, M., Webb, G. and Cullis, P.R. (1985) Biochim. Biophys. Acta 812, 55–65.
[20] Mrsny, R.J., Volwerk, J.J. and Griffith, O.H. (1986) Chem. Phys. Lipids 39, 185–191.
[21] Pagliaccia, C., Wang, X.M., Tardy, F., Telford, J.L., Ruysschaert, J.M. and Cabiaux, V. (2000) Eur. J. Biochem. 267, 104–109.
[22] Wang, X.M., Wattiez, R., Mock, M., Falmagne, P., Ruysschaert, J.M. and Cabiaux, V. (1997) Biochemistry 36, 14906–14913.
[23] Quertenmont, P., Wolff, C., Wattiez, R., Vanderborght, P., Falmagne, P., Ruysschaert, J.M. and Cabiaux, V. (1999) Biochemistry 38, 660–666.
[24] Laemmli, U.K. (1970) Nature 227, 680–685.
[25] Schagger, H. and von Jagow, G. (1987) Anal. Biochem. 166, 368–379.
[26] Goormaghtigh, E., Cabiaux, V. and Ruysschaert, J.M. (1994) Subcell. Biochem. 23, 405–450.
[27] Cabiaux, V., Brasseur, R., Wattiez, R., Falmagne, P., Ruysschaert, J.M. and Goormaghtigh, E. (1989) J. Biol. Chem. 264, 4928–4938.
[28] Goormaghtigh, E., Cabiaux, V. and Ruysschaert, J.M. (1990) Eur. J. Biochem. 193, 409–420.
[29] Vinion-Dubiel, A.D., McClain, M.S., Czajkowsky, D.M., Iwamoto, H., Ye, D., Cao, P., Schraw, W., Szabo, G., Blanke, S.R., Shao, Z. and Cover, T.L. (1999) J. Biol. Chem. 274, 37736–37742.
[30] de Bernard, M., Burroni, D., Papini, E., Rappuoli, R., Telford, J. and Montecucco, C. (1998) Infect. Immun. 66, 6014–6016.
[31] Ye, D., Willhite, D.C. and Blanke, S.R. (1999) J. Biol. Chem. 274, 9277–9282.
[32] Garner, J.A. and Cover, T.L. (1996) Infect. Immun. 64, 4197–4203.
[33] Quertenmont, P., Wattiez, R., Falmagne, P., Ruysschaert, J.M. and Cabiaux, V. (1996) Mol. Microbiol. 21, 1283–1296.