Structural features of the transmembrane E5 oncoprotein from bovine papillomavirus

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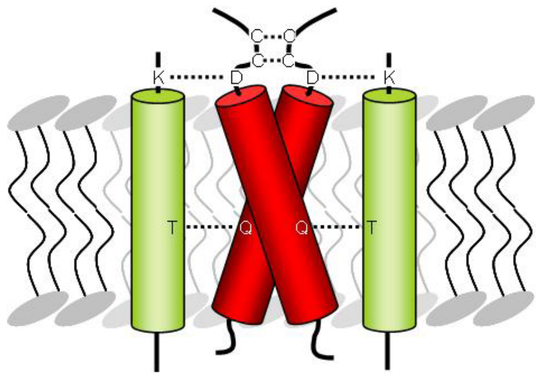
E5 and the PDGF-receptor



Aims

The viral E5 oncoprotein is a small transmembrane protein consisting of 44 mostly hydrophobic residues. As a disulfide-bridged homodimer E5 has been shown to interact with the platelet-derived growth factor receptor (PDGFR-β), resulting in cell proliferation in the absence of the natural ligand. For productive interaction with the receptor it was believed that E5 needs to dimerize via a C-terminal pair of cysteines, although recent data demonstrated that the transmembrane section is capable of dimerization *per se*, in a cysteine-independent manner. The aims of our work are:

- *to elucidate the secondary structure of E5, alone and in complex with the receptor (see poster by S. Hoffmann)*
- *to determine the orientation and alignment of E5 within the membrane, as monomer, dimer, and in complex with the receptor*
- *to investigate the role of the disulfide bridges in dimerization*

Results

Constructs used in our study

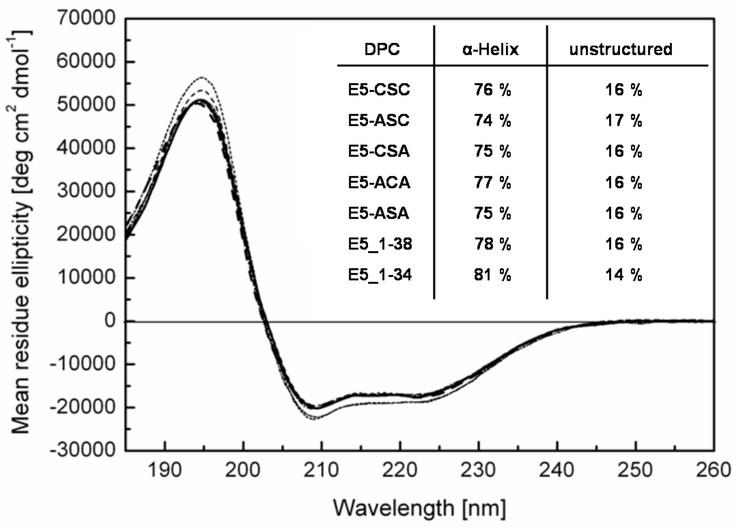
- E5 wildtype protein (= E5-CSC)
- four different cysteine mutants
- two deletion mutants (shortened at the C-terminus)

	1 7 29 37 39 44
E5-CSC	GMPNLWFLLFLGLVAAMQLLLLLFLLLFFLVYWDHFE**C**S**C**TGLPF
E5-ASC	GMPNLWFLLFLGLVAAMQLLLLLFLLLFFLVYWDHFEAS**C**TGLPF
E5-CSA	GMPNLWFLLFLGLVAAMQLLLLLFLLLFFLVYWDHFE**C**SATGLPF
E5-ACA	GMPNLWFLLFLGLVAAMQLLLLLFLLLFFLVYWDHFEA**C**ATGLPF
E5-ASA	GMPNLWFLLFLGLVAAMQLLLLLFLLLFFLVYWDHFEASATGLPF
E5_1-38	GMPNLWFLLFLGLVAAMQLLLLLFLLLFFLVYWDHFE**C**S
E5_1-34	GMPNLWFLLFLGLVAAMQLLLLLFLLLFFLVYWDH

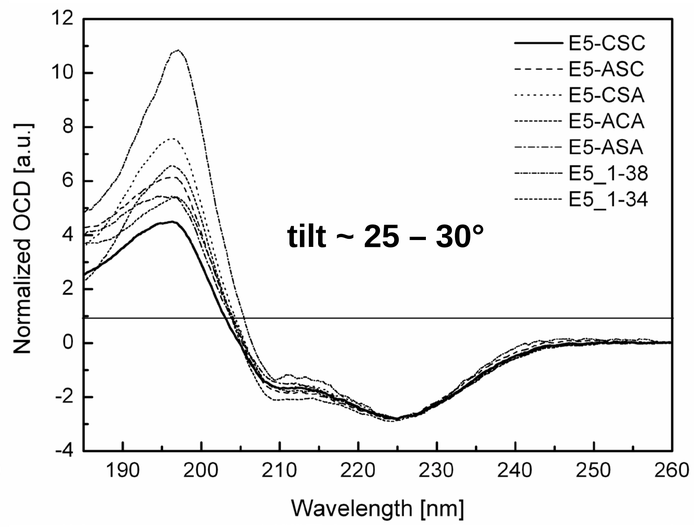
The grey box represents the putative transmembrane segment between Leu7 and Leu29 (predicted by TMHMM and SOSUI algorithm). The cysteine(s) between position 37 and 39 of the different E5-mutants are highlighted and were introduced to probe different ways of dimer association.

Comparison of the secondary structure and orientation of E5 and mutants by CD/OCD

CD/OCD measurements were done to investigate the structure and the orientation of E5 and the mutants in membrane-like environments.



CD spectra of E5-WT and mutants in detergent micelles (10 mM DPC, 20°C). inset: secondary structure deconvolution using CONTIN algorihm.

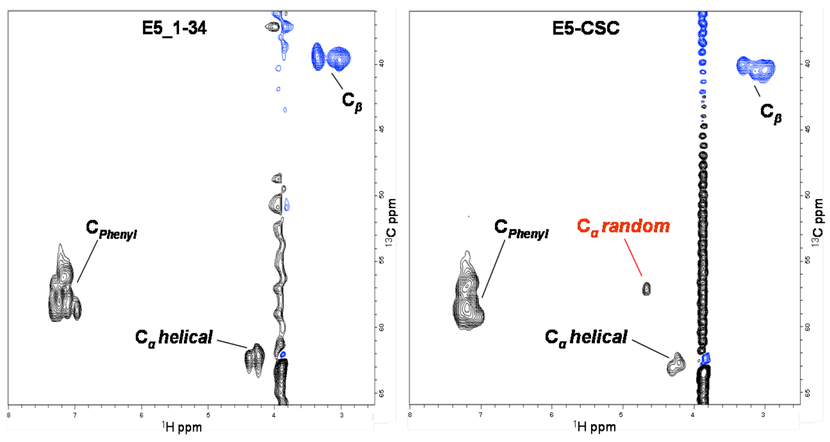


OCD spectra of E5-WT and mutants in aligned lipid bilayers [DMPC/LMPC]. (normalized to the minimum ellipticity at 224 nm).

Chemical shift analysis

A chemical shift analysis was used for a more detailed analysis of the secondary structure of E5. Therefore the wildtype protein and the deletion mutant E5_1-34 were ^{13}C,^{15}N-Phenylalanine labeled and the ^{13}C chemical shifts were measured by HMQC measurements, because they highly dependend on the secondary structure.
E5_1-34 showed only α-helical $^{13}C_\alpha$ shifts, while for the wildtype protein additional random coil shifts were observed, which are caused by Phe 35 and/or Phe 44.

	1 9 33 44
E5-CSC	GMPNLW**F**LL**F**LGLVAAMQLLLLL**F**LLL**FF**LVYWDH**F**ECSCTGLP**F**
E5_1-34	GMPNLW**F**LL**F**LGLVAAMQLLLLL**F**LLL**FF**LVYWDH



HMQC spectra of ^{13}C,^{15}N-Phenylalanine labeled E5-WT and E5_1-34 in 80% deut. TFE, 20% D_2O at 37° C. The C_α, C_β and C_{Phenyl} are marked. The additional peaks at 3,8 ppm in both spectra were background from the solvent.

Comparison of the secondary structure of E5 and mutants by solution NMR

Solution state NMR experiments (HSQC) were done to investigate the influence of the different mutations on the structure of E5. Superposition of the mutant spectra with the E5-CSC wildtype demonstrates that the presence or position of the disulfide bond and the deletions of C-terminal aminoacids has only minor effects on the overall structure, because the majority of the peaks remain unaffected.

Superpositions of the $^1H^{15}N$-HSQC solution NMR spectra of the E5 wildtype protein (black) and mutants (red) in 80% TFE, 20% D_2O at 37° C.

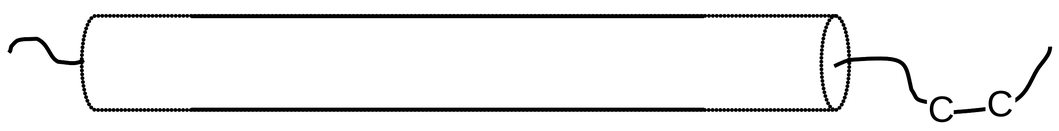
Conclusions

- all E5 variants have a helical secondary structure and a slightly tilted transmembrane orientation
- secondary structure and orientation not affected by cysteine substitutions or deletions of the C-terminal part
- the C-terminal part is probably unstructured
- one disulfide bridge is sufficient for covalent dimerization
- the position of the disulfide bridge is not important
- non-covalent dimerization is also possible via the transmembrane segment

Proposed structural model

Based on our findings we propose a structural model of the E5 protein where the α-helical part is larger than a typical transmembrane helix, as it is flanked by only short unstructured parts at the C- and N-termini. In this model both cysteines lie outside of the helical segment of the protein.

1 7 29 37 39 44
GMPNLWFLLFLGLVAAMQLLLLLFLLLFFLVYWDHFE**C**S**C**TGLPF



Proposed model of the secondary structure of the E5 protein. The grey box represents the originally suggested transmembrane segment between Leu7 and Leu29 from computational structure prediction. The helical part (solid line) of E5 is flanked by distorted helix regions (dashed line). The cysteine-motif lies in the unstructured part located at the terminus.