Structural flexibility of membrane-fusogenic peptides examined by D/H-exchange reactions

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Introduction

SNARE proteins drive different types of intracellular membrane fusion. Recent evidence suggests that their transmembrane segments (TMSs) play a direct role in lipid mixing. Using a FRET-liposome fusion assay, we have previously shown that reconstituted synthetic peptides representing SNARE TMSs are capable of driving membrane fusion in vitro. Interestingly, the fusogenic activity of these peptides is inversely correlated to the stability of their α-helical conformation. This suggested that structural flexibility of SNARE TMSs is important for fusion. Here, we examined the structural flexibility of these peptides via D/H-exchange reactions and mass spectrometry at conditions where they exhibit high α-helix contents.

Experimental Approach

Our goal was to compare the structural flexibility of the wt synaptobrevin TMS to that of two mutants and a oligo-Leu (L16) sequence (Fig. 1), that showed only low fusogenicity (Fig. 2).
CD data showed high helix content from 40 to 100% TFE, whereas syb-wt and syb-multA peptides unfolded at 20% TFE (Fig. 3). D/H-exchange kinetics were done in combination with electrospray ionization mass spectrometry to determine sequence dependent differences.

Peptides

syb-wt	KKKW ILGVICAIILIIIIVY KKK
syb-multA	KKKW I**A**GVI**A**AIILIII**A**VY KKK
syb-L8	KKKW IL**LL**IC**LL**IL**LL**II**LL** KKK
L16	KKKW LLLLLLLLLLLLLLLL KKK

Fig. 1: Design of TMS peptides.

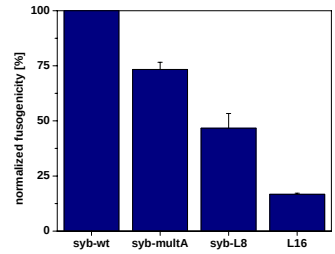

Fig. 2: Relative membrane fusogenicities of our TMS peptides as determined by a fluorescence-based liposome fusion assay.

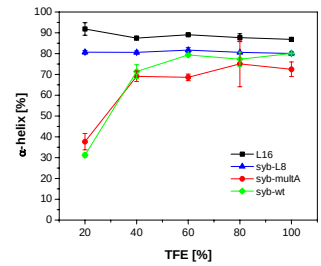

Fig. 3: Dependence of α-helicity as determined by CD-scectroscopy on TFE concentration in aqueous ammonium acetate buffer (pH 7.4).

D/H-exchange experiments

First, the peptides were fully deuterated. Subsequently, they were diluted into protonated solvent. Irrespective of the type of peptide, > 50% of the deuterons exchanged upon dilution into protonated solvent at pH 2 (t = 0 min) suggesting that they are unprotected (Fig. 4, Table I).
The kinetics of protected deuterons depended on the type of peptide investigated (Fig. 5).

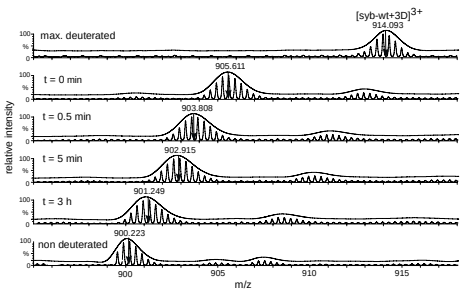

Fig. 4: D/H-exchange of syb-wt peptide at different incubation times. Fully deuterated peptide was diluted into 60% TFE / 40% 10 mM ammonium acetate pH 7.4 at 20.0 °C. The D/H-exchange was stopped by adding formic acid to a final concentration of 0.5 % (v/v).

peptide	D_{max} (theoret.)	D_{max} (measured)	unprotected D (theoret.)	unprotected D (measured)	$\Delta D_{unprot.}$	D after 24 h
L16	38	37.1	19	19.5	0.5	3.4
syb-L8	39	37.9	20	20.4	0.4	0.8
syb-multA	39	38.2	20	22.0	2.0	0.2
syb-wt	40	38.6	21	23.2	2.2	0.6

Table I: Statistics of D/H-exchange

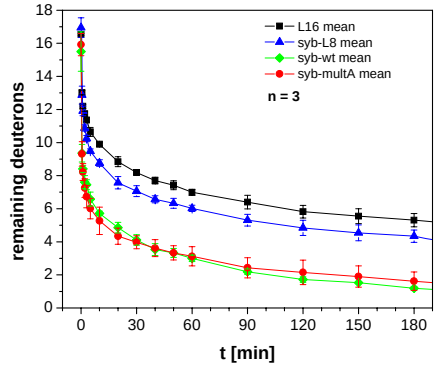

Fig. 5: D/H-exchange kinetic of protected deuterons.
The curves were fitted to the equation:
$D(t) = A \cdot e^{(-k_a \cdot t)} + B \cdot e^{(-k_b \cdot t)} + C \cdot e^{(-k_c \cdot t)}$
where A, B and C represent the number of deuterons, which exchange with the corresponding exchange rate constants k_a, k_b and k_c.

Data evaluation revealed that the different peptides are distinguished by their relative contents of low, medium and fast deuterons (Fig. 6).

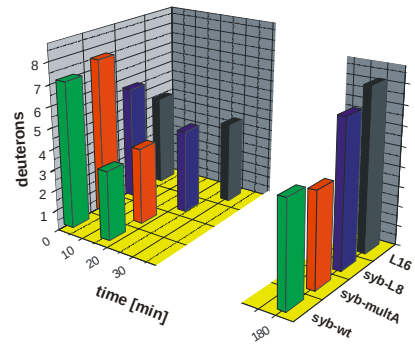

Fig. 6: Comparison of slow, medium and fast deuterons

Slow and fast deuterons exhibit exchange rate constants that depend on the type of peptide (Fig. 7).

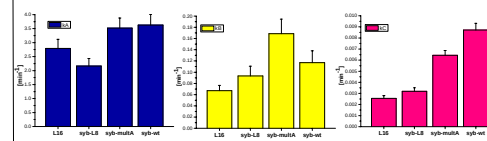

Fig. 7: Comparison of k_a, k_b and k_c rate constants

Conclusions

- **The structural flexibility of membrane fusogenic peptides is reflected by their D/H-back exchange kinetics.**
- **Interestingly, those peptides that exhibit higher membrane fusogenicity display:**
  - **higher numbers of rapidly exchanging deuterons and**
  - **higher exchange rate constants of their "slow" deuterons.**

References

1. Hofmann, M. W., Weise, K., Ollesch, J., Agrawal, A., Stalz, H., Stelzer, W., Hulsbergen, F., deGroot, H., Gerwert, K., Reed, J., and Langosch, D. (2004) De novo design of conformationally flexible transmembrane peptides driving membrane fusion, *Proc Natl Acad Sci U S A 101,* 14776-14781.
2. Langosch, D., Crane, J. M., Brosig, B., Hellwig, A., Tamm, L. K., and Reed, J. (2001) Peptide mimics of SNARE transmembrane segments drive membrane fusion depending on their conformational plasticity, *J. Mol. Biol. 311*, 709-721.