[BIO20] Structural analysis of peptides that interact with Newcastle disease virus

Chia Suet Lin[1], Tan Wen Siang[1], Khozirah Shaari[2], Seetharama D.S. Jois[3] and Khatijah Yusoff[1]

[1]Department of Microbiology, Faculty of Biotechnology and Biomolecular Sciences; [2]Department of Phytomedicine, Institute of Bioscience; and [3]Department of Pharmacy, Faculty of Science, National University of Singapore.

Introduction

Newcastle disease virus is a negative-polarity, single-stranded RNA virus (Lamb and Kolakofsky, 1996). It causes an economically important disease called Newcastle disease. There are three pathotypes of the virus strains, namely, lentogenic, mesogenic, and velogenic, which cause mild respiratory disease to severe intestinal lesions or neurological disease (Alexander, 1997).

NDV initiates infection by attachment of the haemagglutinin-neuraminidase (HN) protein to the cellular membrane and fusion with the fusion (F) protein (Lamb and Kolakofsky, 1996). These proteins have been the sites of inhibition by synthetic peptide inhibitors produced by several groups (Lambert *et al.*, 1996; Young *et al.* 1997; 1999). Ramanujam *et al.* (2002; 2004) successfully discovered a peptide sequence, TLTTKLY, which not only inhibited the propagation of the virus in embryonated chicken eggs but it was also capable of differentiating the different pathotypes of the virus.

In this study, we determined the key amino acid residue of the peptide, CTLTTKLYC, is involved in the peptide-virus interaction. The structures of this peptide determined by circular dichroism, nuclear magnetic resonance, and molecular modelling are also presented.

Materials and Methods

Propagation and purification of NDV

The propagation and purification of NDV were adapted from Ramanujam *et al.* (2002).

Oligonucleotide-directed mutagenesis

Oligonucleotide-directed mutagenesis was adapted from Sambrook *et al.* (2001). Primers were designed for in situ mutagenesis. Uracil-containing ssDNA was prepared by growing the recombinant bacteriophage M13 in *E. coli* strain CJ231. Competent *E. coli* strain TG1 cells were transfected with mutated DNA by a brief heat shock. Plaques formed were picked randomly and DNAs were extracted for sequencing as described in Sambrook *et al.* (2001).

Large-scale preparation of phage

The mutated M13 bacteriophage was prepared in large-scale according to the method described by Sambrook *et al.* (2001). The bacteriophage was amplified in *E. coli* strain ER2738.

Peptide-NDV studies

Experiment for binding capability study was adapted from the methods described by Ramanujam *et al.* (2002). Colour development was done by adding ABTS dissolved in ABTS buffer and optical density was determined by using a microplate reader (BioRad) at λ= 405 nm.

Peptide Purity Determination

Synthetic linear and cyclic (disulfide-constraints) peptide CTLTTKLYC were purchased from Peptron (Korea).

Circular Dichroism (CD) Spectroscopy

The CD experiments were carried out using the JASCO-J715 instrument. Both linear and cyclic peptides were firstly dissolved in Dimethylsulfoxide (DMSO) (0.5% v/v) and then diluted to respectively concentrations with different solvents. Spectra were collected from 260 nm to 190 nm with a cylindrical quartz cell of path length 1 mm.

NMR spectroscopy

The peptides were dissolved in 100% deuterated DMSO. The one- and two-dimensional NMR experiments were carried out using the 300 MHz Bruker DPX spectrometers with a variable temperature probe. Total correlation spectroscopy

(TOCSY) (Bax and Davis, 1985b), double quantum-filter COSY (DQF-COSY) (Rance *et al.*, 1983), nuclear overhauser enhancement (NOESY) (Kumar *et al.*, 1981) and rotating frame nuclear overhauser enhancement (ROESY) (Bax and Davis, 1985a) experiments were carried out by pre-saturation of water during relaxation delay. Data were collected by the time-proportional phase increment (TPPI) method (Marion and Wüthrich, 1983). The NMR spectra were processed using xwinnmr software (Bruker, Inc., USA). Coupling constants ($^3J_{HN\alpha}$) were measured from the DQF–COSY spectra, and dihedral angles ϕ were calculated from the coupling constants (Bystrov, 1976). NOE cross peaks were assigned for $d_{N\alpha}(i, i)$, $d_{\alpha N}(i, i+1)$, and $d_{NN}(i, i+1)$.

Computational Methods

Conformational space was searched using the Discover program version 2000. Calculations were performed on an Origin 2000 Silicon Graphics computer using consistent valence force field (Cvff). NOEs were used in the calculations. Linear peptide was constructed and it was cyclized using NOE and disulfide bond constraints by running MD simulations *in vacuo*. The disulfide bond was formed to make the cyclic structure. This structure was then subjected to simulated annealing procedure (Sutcliffe, 1993) as described (Jois, 1999).

The structures obtained were subjected to high temperature MD simulations *in vacuo*. Final structures were chosen and subjected to molecular dynamics again. The resulting structures were further energy minimized with steepest descent methods and conjugate gradient method. The conformations of the peptide that is consistent with the NMR data were chosen as a representative structure.

Results

Phage-NDV Binding Study

All mutants obtained were used to interact with NDV. Wild type M13 (KE) phage was used as a negative control whilst phage-bearing the CTLTTKLYC (TL) sequence represents the positive control. All mutants exhibited a slight increment in their binding capability compared to that of the original peptide except mutant Y8A and K6R-Y8A.

CD Studies

Cyclic peptide was determined to acquire type I β-turn. The CD spectra of the linear peptide show that there are mixtures of peptide secondary structures in solution.

NMR Spectroscopy

Assignments of the ^1H resonances were achieved by procedure described by Wüthrich (1983). The NMR data obtained suggests that the cyclic peptide exhibiting a turn structure along with β-strain type conformation (Wüthrich, 1983).

Discussion

C1, K6 and Y8 of the CTLTTKLYC sequence were substituted with alanine. Replacement of Y8 with A severely disrupted the phage-NDV interaction but replacement with F did not affect the binding capability. The aromatic ring of Y is believed to be involved in a non-covalent interaction between the peptide and the surface proteins of NDV.

The positively charged residue, K6, was not involved in the interaction. Substitution of K6 with A or R did not significantly affect the binding capability of the peptide. Residues C1 was substituted with A to break the disulfide bond with C9 and subsequently affect the binding capability of the peptide. Surprisingly, the binding capability of the mutant was not affected suggesting that the structural rigidity of the peptide is not necessary.

The secondary structure was initially determined by using CD and the data obtained subsequently aided in tertiary structure determination by using NMR and molecular modelling. Most of the conformers generated using molecular modelling exhibited β-turns. Two structures were obtained: the first structure exhibit β-turn and distorted β-turn, while the second structure exhibit extended structure. The presence of the β-turn is supported by the observation of NOE between the NH of the amino acid residues.

The dual-structure of the peptides could be the reason that there are two K_d^{rel} values for the peptide obtained from previous experiments Ramanujam *et al.* (2002, 2004).

Acknowledgements

The authors wished to thank the Ministry of Science, Technology and Innovation (MOSTI), Malaysia for the National Acknowledgements. This study was supported by IRPA grants 09-02-04-0753-EA001 and 01-02-04-003 BTK/ER/006 of the Ministry of Science, Technology and Innovation, Malaysia.

References

Alexander, D. J. (1997). Newcastle disease and other avian *Paramyxoviridae* infection. *In* B. W. Caineck (Ed.), Diseases of Poultry 10th Edn. pp 541-569: Amex, IA, Iowas State University Press.

Bax, A., and Davis, D. G. (1985a). Practical aspects of two-dimensional transverse NOE spectroscopy. *Journal of Magnetic Resonance* **63**: 207-213.

Bax, A., and Davis, D. G. (1985b). MLEV-17-based two-dimensional homonuclear magnetization transfer spectroscopy. *Journal of Magnetic Resonance* **65**: 355-360.

Bystrov, V. F. (1976). Spin-spin coupling and the conformational states of peptide systems. *Progress in NMR Spectroscopy* **10**: 41-81.

Hernandez, L. D., Hoffman, L. R., Wolfsberg, T. G., and White, J. M. (1996). Virus-cell and cell-cell fusion. *Annual Review Cell Biology* **12**: 627-661.

Hruby, V. J., Al-Obeidi, F., and Kazmierski, W. (1990). Emerging approaches in the molecular design of receptor-selective peptide ligands: conformational, topographical and dynamic considerations. *Biochemistry* **268**: 249-262.

Jois, S. D. S., Tibbetts, S. A., Chan, M. A., Benedict, S. H. and Siahaan, T. J. (1999). A Ca^{2+} binding cyclic peptide derived from the α-subunit of LFA-1:inhibitor of ICAM-1/LFA-1-mediated T-cell adhesion. *Journal of Peptide Research* **53**:18-29.

Kumar, A., Wagner, G., Ernst, R. R., and Wüthrich, K. (1981). Buildup rates of the nuclear overhauser effect measured by two-dimensional proton magnetic resonance spectroscopy. *Journal of American Chemistry Society* **103**: 3654-3658.

Lamb, R. A. and Kolakofsky, D. (1996). *Paramyxoviridae*: The viruses and their replication *in*: Fields virology, 3rd edn. Fields, B. N., Knipe, D. M., Howley, P. M. (ed). pp. 1177-1204. Philadephia: Lippincott-Raven.

Lambert, D. M., Barney, S., Lambert, A. L., Guthrie, K., Medinas, R., Davis, D.E., Bucy, T., Erickson, J., Merutka, G., and Petteway, S.R., Jr. (1996). Peptides from conserved regions of paramyxovirus fusion (F) proteins are potent inhibitors of viral fusion. *Proceeding National Academy of Science, USA* **93**: 2186-2192.

Marion, D., and Wüthrich, K. (1983). Application of phase sensitive two-dimensional correlated spectroscopy (COSY) for measurements of ^{1}H-^{1}H spin-spin coupling constants in proteins. *Biochemical, Biophysical Research Communications* **113**: 967-974.

Ramanujam, P., Tan, W. S., Nathan, S., and Yusoff, K. (2002). Novel peptides that inhibit the propagation of Newcastle disease virus. *Archives of Virology* **147**: 981-993.

Ramanujam, P., Tan, W. S., Nathan, S., and Yusoff, K. (2004). Pathotyping of Newcastle disease virus with a filamentous bacteriophage. *Biotechniques* **36**: 296-300.

Rance, M., Sorensen, O. W., Bodenhausen, G., Wagner, G., Ernst, R. R. and Wüthrich, K. (1983). Improved spectral resolution in COSY ^{1}H NMR spectra of protein via double quantum filtering. *Biochemical, Biophysical Research Communications* **117**: 479-485.

Sambrook, J. and Russel, D.W. (2001) Molecular cloning. A laboratory manual, 3rd edn. Cold Spring Harbor Laboratories Press, Cold Spring Harbor.

Sutcliffe, M. J. (1993). Structure determination from NMR data II. Computational approaches. *In*: Roberts, G. C. K., *NMR of Macromolecules*: A practical approach. pp 359-390. New York: Oxford University Press

Wüthrich, K. (1983). NMR of proteins and nucleic acids. New York: John Wiley & Sons.Young, J. K., Hicks, R. P, Wright, G. E., and Morrison, T. G. (1997). Analysis of a peptide inhibitor of paramyxovirus (NDV) fusion using biological assays, NMR, and molecular modelling. *Virology* **238**: 291-304.

Young, J. K., Li, D., Abramowitz, M.C., and Morrison, T. G. (1999). Interaction of peptides with sequences from the Newcastle disease virus fusion protein heptad repeat regions. *Journal of Virology* **73**: 5945-5956.