PENERBITAN PEGAWAI

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Abstract

A fusion phage carrying the peptide sequence CTLTTKLYC was selected from a disulfide constrained phage display random peptide library against Newcastle disease virus (NDV) strain AF2240. The binding affinity of the phage for AF2240 is both pH and temperature-dependent with optimal binding occurring at pH 4 and 4°C respectively. No binding was observed at pH 12. Binding decreased with temperatures above 4 °C until it was completely abolished at 80°C. The results suggest that the fusion activity of the virus is promoted in acidic conditions.

Key words: binding affinities, Newcastle disease virus, fusion phage.

Introduction

Newcastle disease virus (NDV) is an avian virus endemic in many countries, particularly in Asia, the Middle East, Africa, Central and South America [1]. This RNA virus contains a lipid bilayer envelope in which the antigenic surface glycoproteins are embedded. These glycoproteins are the haemagglutination-neuraminidase (HN) and fusion (F) proteins. The HN protein possesses haemagglutination (HA) and neuraminidase (NA) activities while the F protein is involved in the fusion activity of the virus [2]. The F protein contains two heptad repeats (HR1 and HR2) and a fusion peptide region which play an important role in fusion. In order for the virus to infect the host cell, the HN protein first binds to a sialic acid-containing receptor which then activates the F protein to undergo a series of conformational changes [3] resulting in the insertion of the fusion peptides into the target membrane. This interaction further stabilizes the HR1 and HR2 six-stranded coil interaction complex [4] which is believed to bring the target membranes within close proximity of each other for further fusion activities.

A fusion phage carrying the peptide sequence CTLTTKLYC was obtained from a disulfide constrained phage display library by affinity selection against the velogenic NDV strain AF2240 [5], first isolated in a field outbreak in the 1960s in Malaysia [6]. The interaction between the phage and AF2240 mimics the interaction between NDV and its receptor; therefore NDV-phage interaction provides a model for NDV-host (cell) interaction. An antibody-phage competition assay showed that the isolated phage competes with polyclonal antibodies raised against NDV for binding on AF2240 [5]. Furthermore, an equilibrium binding assay in solution revealed that there are two possible binding sites for this phage on NDV strains, with relative dissociation constants of 2.8 ± 0.2 pM and 1.4 ± 0.2 nM [7]. Both linear and cyclic forms of the synthetic peptides bearing the peptide sequence carried by the fusion phage inhibited haemolytic activity of the F protein but not the NA or the HA activities of the HN protein of the virus [5]. However, the possibility of the interaction at NA site cannot be disregarded, as the experiment was performed under extreme pH and temperature conditions. These factors affect the structure and conformation of a protein or peptide, as well as peptide-peptide and protein-protein interactions. In the present investigation, the binding of CTLTTKLYC phage with NDV strain AF2240 at different pH and temperatures was studied.

Materials and Methods

AF2240 was propagated and purified as described in Yusoff *et al* [8]. The large-scale propagation and purification of the CTLTTKLYC - carrying phage was done according to the method described by Ramanujam *et al* [5]. Phage titration was carried out according to a method described by Sambrook *et al* [9].

Determination of interaction at different pH levels

NDV strain, AF2240 (20 µg/ml; 100 µl) was coated on microtiter plate wells overnight with Na_2CO_3/$NaHCO_3$

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buffer (29.3 mM Na_2CO_3, 70.7 mM $NaHCO_3$; pH 9.6). The wells were washed 5X with TBST (0.05 M Tris-HCl, 0.15 M NaCl, 0.1 % v/v Tween-20; pH 7.4) followed by blocking with TBS (0.05 M Tris-HCl, 0.15 M NaCl; pH 7.4 and 0.1 % BSA w/v; 120 µl) and 1 h of incubation at room temperature. Stock phage was diluted to 10^{10} pfu/ml with various buffers at different pH (50 mM citrate buffer for pH 2 and 4; 0.1 M sodium phosphate buffer for pH 6 and pH 8, TBS was used for pH 7, and CAPS (3-cyclohexylamino-1-propanesulfonic acid) for pH 10 and 12. Diluted phage was then added into the NDV strain AF2240 - coated wells and incubated for 1 h at room temperature. Wells were washed and numbers of phages bound at different pH were determined by titrating the eluate.

Determination of interaction at different temperature

Microtiter plate wells were coated with NDV strain AF2240 and blocked as described above. Stock phage was diluted in TBS to a concentration of 10^{10} pfu/ml. These phages were incubated at different temperatures (4-80 °C) for 1 h. The treated phage particles were immediately added into the NDV - coated wells and incubated for 1 h at room temperature. The amount of phage bound to NDV was determined by titrating the eluted phage.

Results and Discussion

The fusion peptide showed the highest binding at pH 4 and relatively high binding at pH 7 and 8 (Figure 1). It is possible that the conformation of the peptide that is manifested at pH 4 may be the best-suited form for the interaction with NDV, thus highest binding was obtained

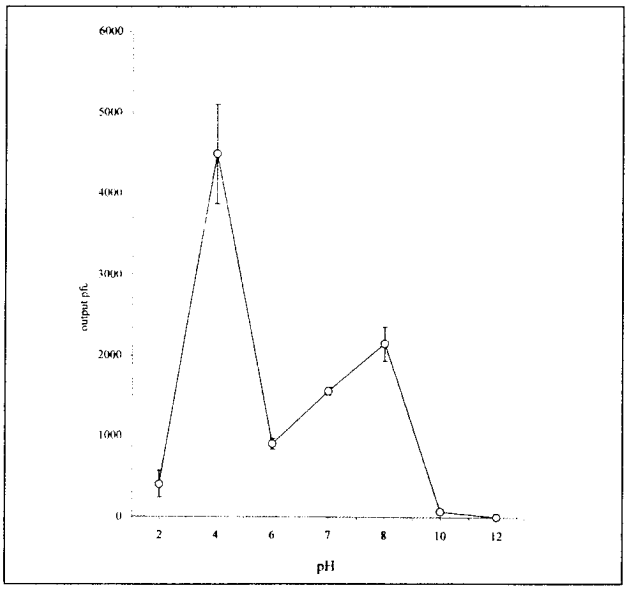

Figure 1: Interaction of TLTTKLY Phage with NDV at Different pH Buffers. Output plaque forming units (pfu) reveals the amount of phage bound to NDV at different pH. Experiments were performed in triplicates and the error bars represent the standard deviation of the mean.

at this pH. The very low binding at pH 2 and 10, and complete inhibition of binding at pH 12 is most likely due to a complete change in the peptide conformation that does no longer fits into its docking site. The peptide selection procedure was performed in a buffer of pH 7.2 [5] and this may explain why pH 7 and 8 may be tolerated by the peptide without much loss of its energy, and relatively high binding was observed.

The molecular structure of a protein or peptide is determined by the presence of the ionisable side chains it contains. Under different pH conditions these ionisable groups may be involved in the stabilisation of its molecular structure resulting in loss of energy. Furthermore, it is also possible to have conformational changes which may affect the activity of the peptide or protein [10]. When a ligand interacts with a protein at a specific binding site, part of the free energy change in the binding includes free energy of the conformational adjustment of the protein.

The change in pH affects not only the structure and conformation of the fusion peptide but most likely the fusion peptide binding site on the surface of the virus. It has been suggested that for many viruses, exposure to low pH after receptor binding triggers conformational changes on the glycoproteins to promote fusion activity [11]. In the present experiment, the use of a pH 2.2 elution buffer minimises the interaction between the fusion phage and AF2240.

The CTLTTKLYC peptide contains 3 Thr residues, with an uncharged polar side chain; 2 Leu residues, with an aliphatic nonpolar hydrocarbon side chain; 1 Tyr residue, with an uncharged polar phenolic side chain, and 1 Lys residue that is a basic amino acid with a positively charged side chain. The cysteine residues flanking the peptide contain an uncharged polar side chain thiol group which forms a disulfide bond. In the presence of the disulfide bond, the polar and non-polar residues and the positively charged Lys, the peptide possesses a defined structure and conformation. The pK value for the Lys is 10.54 and when the pH value is lower than the pK, the peptide is positively charged. With the change in the pH of its surroundings, the probability of this peptide altering its structure or conformation to stabilize its form is relatively high. Therefore the interaction of either the peptide with NDV or both will be affected resulting in the display of variation in its binding affinity. The paramyxovirus F protein does not require the acidic pH of endosomes to activate fusion activity [12]. Therefore the extreme pH used in the neuraminidase activity test would have altered the structure and the conformation of either of the interacting molecules thus, displaying the observed results as stated.

Figure 2 shows that with an increase in the temperature, the number of TLTTKLY phage bound to NDV reduced gradually. The interaction was completely abolished for

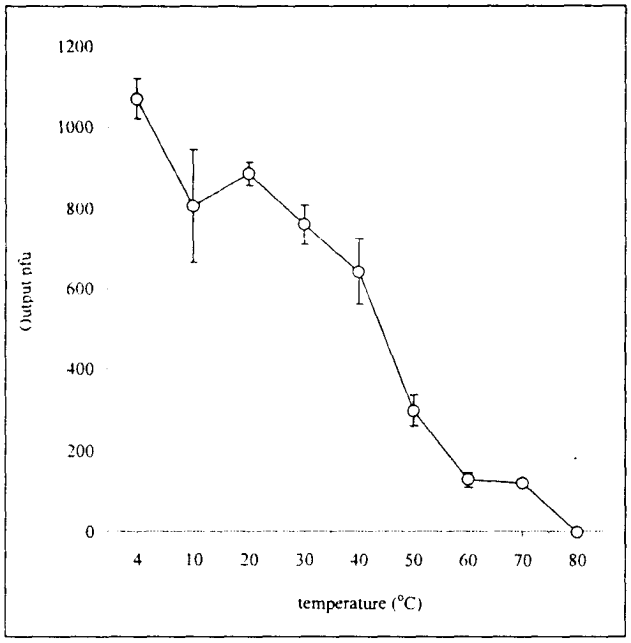

Figure 2: Interaction of TLTTKLY Phage with NDV at Different Temperatures. Experiments were performed in triplicates and the error bars represent the standard deviation of the mean.

fusion peptide exposed to 80 °C. Temperature plays an important role in the stability of peptides or proteins. Some peptides may be denatured by high temperatures, or in some cases by temperatures only a few degrees higher than that in their native environment [13]. There was a marked drop in the number of phages interacting with AF2240 between temperatures 40 °C to 50 °C. No interaction was detected at 80 °C. It is possible that exposure to heat distorted the structure or the conformation of the fusion peptide, which in turn abolished the interaction between the two molecules thus displaying the observed binding profile.

In conclusion, data obtained indicate that the optimal pH for NDV-phage interaction is pH 4 and the phage is most stable at 4 °C. These data may be useful for the development of a diagnostic test to detect NDV with the fusion phage.

Acknowledgement

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