Anomalous Behaviour of Newcastle Disease Virus Haemagglutinin–Neuraminidase Protein in Western Blotting Analysis of Monoclonal Antibody Binding Sites

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SUMMARY

Previous studies with a particular monoclonal antibody (MAb 445) raised against the Ulster strain of Newcastle disease virus (NDV) have shown that this MAb immune-precipitates only the 74000 mol. wt. (74K) haemagglutinin–neuraminidase protein (HN) of both Ulster and Beaudette C strains of NDV. Using the technique of Western blotting, it is shown that under certain denaturing conditions virion proteins co-migrating in SDS–polyacrylamide gels with the faster migrating 67K fusion protein and 53K nucleocapsid/nucleocapsid-associated protein, as well as the 74K HN, all reacted strongly with MAb 445 and with MAb 14 which is also directed against the HN protein. Analysis of this anomalous behaviour using SDS–polyacrylamide diagonal gel electrophoresis has led to the unexpected conclusion that the 74K HN can electrophorese as three immunoreactive species of apparent mol. wt. 74K, 67K and 53K. If the Western blotting technique is to be applied to proteins which have not been sufficiently denatured (in an attempt to preserve epitope integrity) it is important to establish that no additional proteins remain associated with the protein bands that react with the MAb.

Monoclonal antibodies (MAbs) are particularly useful in detecting and purifying individual virus proteins because a given MAb should only react with an epitope unique to a particular protein. Various methods can be used to determine to which protein a given MAb is directed; immunoprecipitation and Western blotting (Burnette, 1981) are the most commonly used techniques.

MAbs 445 and 14, raised against Newcastle disease virus (NDV) (Russell et al., 1983a, b), immune-precipitate only the 74000 mol. wt. (74K) haemagglutinin–neuraminidase protein (HN) from NDV strains (Russell et al., 1983b) and have been used to select independent mutants of the Beaudette C strain resistant to neutralization by MAb 445 and MAb 14. All of these mutants have an altered HN (revealed by isoelectric focusing gel electrophoresis) (Samson et al., 1985; and unpublished data). In an attempt to use the technique of Western blotting to investigate further the properties of a temperature-sensitive (but still MAb 445- and MAb 14-sensitive) Beaudette C mutant ts53 [which had been shown earlier to possess a thermolabile HN (Harper et al., 1983)], it was found that three proteins from wild-type virions reacted with either MAb 445 or 14 but only the expected HN reacted from the ts53 virions.

The original anomalous behaviour of wild-type HN was noticed in an experiment designed to see whether the known thermolability of HN in ts53 virions [measured by both haemagglutination and neuraminidase activity of intact virions (Harper et al., 1983)] compared to wild-type was reflected in the reaction of HN proteins to anti-HN MAbs following Western blotting of virions disrupted in SDS-containing buffer at different temperatures and separated by SDS–PAGE.
Fig. 1 shows the effect of heating purified virions of NDV Beaudette C wild-type and ts53 in a sample buffer (Laemmli, 1970), modified to contain only 0.1% (w/v) SDS and no mercaptoethanol or other reducing agent, on the pattern of MAb 445 and 14 binding by Western blotting. Only wild-type virions disrupted in sample buffer at 34 °C showed the antibody-binding triplet banding pattern corresponding to the migration positions of HN, fusion protein (F) and nucleocapsid/nucleocapsid-associated protein (NP/NAP) (Samson et al., 1981) (Fig. 1b, c). Heating the wild-type virion preparation in the SDS buffer at 50 °C for 30 min prior to SDS-PAGE destroyed the binding reactions associated with the F and NP/NAP regions and increased the amount of binding at the expected slower migrating HN position. The ts53 mutant only showed a binding reaction in the expected HN position whether heated at 50 °C or not. (Some non-specific binding could be seen in the NP/NAP region when high levels of protein were loaded on to the gels.)

Several explanations could account for the unusual behaviour of the wild-type virions: (i) MAb 445 and MAb 14 may be polyclonal and not monoclonal and epitopes on two of the three proteins may be destroyed by heating, (ii) all three proteins may possess a common epitope which binds MAb 445 and 14 and two of these epitopes may be destroyed by heating, (iii) the
epitopes recognized by MAb 445 and 14 may lie only within HN, but this protein may have been fragmented at some stage and the epitopes possibly trapped by two other virion proteins (this trapping is relaxed when the virions are heated), (iv) the epitopes recognized by MAbs 445 and 14 may lie only within HN but a proportion of the virion’s intact HN possibly migrates faster than normal on SDS–PAGE and co-electrophoreses with other virion proteins (this abnormal migration is destroyed when the virions are heated).

The first explanation is very unlikely because of the way in which MAbs are selected and re-cloned several times. In addition, immunoprecipitation assays using radioactive proteins made in chick embryo fibroblasts following infection of the Ulster or Beaudette C strains of NDV and precipitated with either rabbit anti-mouse Ig or Staphylococcus aureus cells respectively, precipitate only HN (74K) (Russell et al., 1983b; Samson et al., 1985). Finally, MAb 445 fails to bind HN from MAb 445-resistant mutants of Beaudette C when assayed by immunoprecipitation and these mutants show an altered isoelectric point only in HN (Samson et al., 1985).

To distinguish between the possible explanations, two different experiments were conducted. The first was to partially purify NDV glycoproteins (HN and F) and separate them from nucleocapsids (containing N and NAP) and matrix (M) protein. The method used was a modification of that of Scheid & Choppin (1973). As shown in Fig. 2, glycoproteins almost devoid of nucleocapsids and M protein still exhibited the triple banding pattern characteristic of unheated SDS-disrupted whole virions. Glycoprotein-free (pellet) preparations showed no significant binding which indicates that the antibody-reactive species co-migrating with NP/NAP must be derived from glycoprotein only.
Short communication

Fig. 3. Diagonal SDS–PAGE analysis of NDV virion proteins. Individual well sections cut from the first dimension SDS–polyacrylamide gel run (Fig. 1) containing purified wild-type virions which had either been heated (HT) or unheated (UN) at 50 °C were stored overnight at 4 °C prior to equilibration in modified Laemmli sample buffer for 30 min at 50 °C. The sections were then loaded on top of a set of four Laemmli 10% SDS–polyacrylamide slab gels and electrophoresed as in Fig. 1. One pair of slab gels was stained in Coomassie Brilliant Blue (a, c). The other pair (b, d) was electrophoretically blotted to sheets of nitrocellulose incubated with MAb 445, then with conjugate and substrate as described in Fig. 1. Virions for (a, b) were heated at 50 °C for the first dimension (and second dimension); virions for (c, d) were unheated for the first dimension and heated prior to the second dimension.

In the second type of experiment, samples of wild-type virions were either heated in modified sample buffer at 50 °C for 30 min or kept at 34 °C for 10 min before cooling in ice/water prior to running in an SDS–polyacrylamide slab gel. Portions of the slab gel were taken for staining in Coomassie Brilliant Blue, for electrophoretic blotting to a nitrocellulose filter and also individual sample well gel sections were cut out for electrophoresis in a second dimension slab gel. These individual sections were incubated in a modified sample buffer at 50 °C for 30 min prior to electrophoresis in the second dimension (Samson et al., 1980).

Fig. 3 (a, b) shows the effect of heating wild-type virions in modified sample buffer prior to the first dimension SDS–PAGE, followed by equilibration of the first dimension gel section in sample buffer at 50 °C prior to the second dimension SDS–PAGE. As expected, only one MAb 445-binding spot was revealed on the nitrocellulose blot (Fig. 3b) corresponding to the position of the Coomassie Brilliant Blue-stained HN (Fig. 3a). In contrast, wild-type virions that were not heated prior to the first dimension, but were treated at 50 °C prior to the second dimension SDS–PAGE revealed a more complex spot pattern. Three MAb 445-binding spots were revealed on the nitrocellulose blot (Fig. 3d) all of which had the migration characteristics of HN in the second dimension. The migration characteristics in the first dimension, however, were HN-, F- and NP/NAP-like (compare with Fig. 3c and Fig. 1).
These observations discount the hypothesis that small fragments of HN bearing the MAb 445 epitope co-migrate with other virion proteins and clearly show that a proportion of intact HN proteins can, under certain denaturing conditions in SDS, exhibit a greater relative mobility on SDS-PAGE than heated HN. There is therefore no need to invoke other hypotheses for this anomalous behaviour.

The question of why only a subfraction of wild-type HN molecules exhibits this phenomenon and why the ts53 mutant does not, remains an intriguing mystery. Clearly, if Western blotting is to be applied to material which has not been fully denatured (in an attempt to preserve epitope structure), if more than one protein band reacts with a MAb, then it is important to establish that no additional proteins remain associated with the protein bands that react with the MAb.

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REFERENCES


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