Distribution of B-cell epitopes on the pseudorabies virus glycoprotein B

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In order to map antigenically important regions of glycoprotein B (gB) of pseudorabies virus (PrV), a panel of recombinant fragments of gB expressed in E. coli and truncated fragments of gB generated by cleavage of purified native gB with trypsin and cyanogen bromide was analysed by using 26 monoclonal antibodies directed against gB. Three continuous epitopes were localized in the vicinity of the N terminus of gB, between amino acids (aa) 59 and 126. One continuous epitope mapped between residues 214 and 279. The residues involved in the assembly of eight discontinuous epitopes were located between aa 540 and 734. The constituents of two discontinuous epitopes were harbourd in a segment encompassing aa 540–646. The clustering of continuous epitopes at the extreme N terminus of PrV gB and the locations of residues involved in the assembly of discontinuous epitopes of PrV gB are in good agreement with data on epitope locations in gB homologues from other herpesviruses.

Glycoprotein B (gB) of pseudorabies (Aujeszky’s disease) virus (PrV), a major constituent of the viral envelope, consists of 913 amino acids (aa), including a 58 aa cleavable signal peptide (Robbins et al., 1987; Whealy et al., 1990). The glycoprotein undergoes glycosylation and cleavage between Arg-502 and Ser-503 during transport through the exocytic pathway (Whealy et al., 1990). The glycoprotein exists as a disulphide-linked complex of three glycoproteins, gBa, gBb and gBc; the gBb and gBc subunits are the cleavage products of gBa (Hampl et al., 1984; Lukács et al., 1985). PrV gB belongs to the family of highly conserved herpesvirus glycoproteins (Robbins et al., 1987) that are implicated in the processes of virus entry and cell-to-cell spread (Rauh & Mettenleiter, 1991; Peeters et al., 1992). PrV gB plays an important role in inducing protective immunity (Marchioli et al., 1988; Riviere et al., 1992; Nakamura et al., 1993; Mengeling et al., 1994; Xuan et al., 1995) and is considered to be a promising candidate for a subunit vaccine.

At present, there are few data concerning the distribution of antigenic and immunogenic regions throughout the PrV gB molecule. Recently, by using a panel of 26 monoclonal antibodies (MAbs) directed against PrV gB, we mapped four continuous epitopes in the gBb subunit and a cluster of 10 overlapping discontinuous epitopes that were assembled, at least partially, from residues located in the gBc subunit (Zaripov et al., 1998). In this report, we describe the localization of continuous epitopes and regions involved in the assembly of discontinuous epitopes of PrV gB. Recombinant fragments of gB expressed in E. coli, as well as truncated forms of native gB generated by trypsin and cyanogen bromide (CNBr) cleavage, were used to map antibody-binding sites in the gB molecule.

A set of recombinant DNA constructs encompassing gB sequences of PrV Ka strain was generated by using the pGEX-3X expression plasmid (Pharmacia) as a vector. The DNA fragment encoding gB sequences between aa 86 and 480 was produced by cloning the XhoI fragment of the gB gene into the SmaI site of pGEX-3X. By treating this construct with different restriction endonucleases, a number of truncated recombinant constructs were produced (86–416, 86–326, 86–279, 86–214 and 86–115). PCR was used to clone DNA fragments that encode gB sequences covering aa 59–126, 428–502 and 540–734. The recombinant construct encoding the sequence between aa 540 and 646 was generated from construct 540–734 by using restriction endonuclease ScaI. The recombinant proteins obtained covered the entire sequence of the gBb subunit; the gBc subunit was represented by two segments encompassing aa 540–646 and 540–734, which correspond to a prominent part of the putative ectodomain of gBc (Fig. 1b). Recombinant proteins were expressed in E. coli as fusions with...
glutathione S-transferase (GST) from *Schistosoma japonicum*. The recombinant proteins were analysed by Western blot as described previously (Morenkov et al., 1997) with a previously characterized panel of 26 MAbs directed against gB (Zaripov et al., 1998). Previously, we showed that MAbs specific for discontinuous epitopes of gB retained reactivity against viral gB in a Western blot, although at a relatively low level, even after boiling the samples under reducing conditions before SDS–PAGE (Zaripov et al., 1998). Therefore, we expected that *E. coli*-expressed recombinant fragments, which presumably
lack authentic higher-order structure (Cason, 1994), could be used to map both continuous epitopes and the regions involved in the assembly of discontinuous epitopes.

Fig. 1(a, c) shows the results of SDS–PAGE (Laemmli, 1970) of these recombinant proteins and the patterns of reactivity with representative MAbs. The results of these experiments can be summarized as follows. (i) MAb 79/9, specific for the continuous epitope of gBb, only reacted with the fusion protein containing aa 59–126. Therefore, its epitope is located in the region of the first 67 amino acids of mature gB. (ii) MAb 21/2, directed against an epitope that is not exposed in virion-associated PrV gB, showed reactivity with all fusion proteins except those lacking aa 214–279. This indicates that these residues are the boundaries of the MAb 21/2 epitope. (iii) MAbs 24/11 and 34/2, directed against two non-overlapping discontinuous epitopes, recognized recombinant proteins encompassing aa 540–734 and 540–646. Therefore, the constituents of the epitopes of MAbs 24/11 and 34/2 were mapped between aa 540 and 646. (iv) Twenty MAbs specific for eight discontinuous epitopes only showed reactivity with the fusion protein comprising aa 540–734, which suggests that this segment contains the residues involved in the formation of these discontinuous epitopes.

MAbs 13 and 86/15, specific for continuous epitopes of gBb, failed to recognize any of the recombinant proteins. The locations of these epitopes were deduced from the following experiments performed with affinity-purified PrV gB (Zaripov et al., 1998).

Limited trypsin digestion of gB resulted in the generation of a gBb fragment shortened by approximately 15 kDa that lost reactivity with MAbs 13, 79/9 and 86/15 (unpublished results). Therefore, the possible locations of the binding sites of MAbs 13 and 86/15 were restricted to N- and/or C-terminal segments of gBb no more than 15 kDa in size. Cleavage of gB with CNBr (Gross, 1967) gave rise to two large gBb fragments, 56–60 and 37 kDa in size, which reacted with MAbs 79/9, 13 and 86/15, as revealed by Western blot (Fig. 2). MAB 21/2, which mapped between aa 214 and 279, showed a different pattern of reactivity with CNBr-derived fragments of gBb (Fig. 2, lane 8). In view of the location of the MAb 79/9 epitope at the N terminus (see above), the only possible explanation for the reactivity of all three MAbs with a 30 kDa-truncated gBb fragment (37 kDa fragment) is colocalization of the corresponding epitopes in one part of the gBb molecule, namely at the N terminus. If the epitopes of MAbs 86/15 or 13, or both, resided in the C-terminal part of gBb, this epitope(s) would inevitably be missing in the 37 kDa fragment that contains the epitope of MAb 79/9. Therefore, these data clearly indicate that the epitopes of MAbs 13 and 86/15 are in an N-terminal fragment of gBb no more than 15 kDa in size.

It is noteworthy that storage of affinity-purified gB in solution led to a slow degradation of the glycoprotein to a form that was recognized on a Western blot by MAb 79/9 but not by MAbs 86/15 and 13; in this case, the epitopes of MAbs 13 and 86/15 were lost from both the gBa and gBb subunits without any detectable decrease in the molecular masses of the truncated subunits (not shown). This indicates that the epitopes of MAbs 86/15 and 13 are located upstream of the MAb 79/9 epitope, at the extreme N terminus of gBb.

Fig. 3 is a schematic topographical map of the PrV gB epitopes. Three continuous epitopes were mapped at the extreme N terminus of gB, in the segment that encompasses aa 59–126. The amino acid sequence in this region is compatible with high flexibility, accessibility and hydrophilicity, features characteristic of antibody-binding sites. It should be noted that the clustering of linear epitopes in the vicinity of the N terminus is a distinguishing feature of herpesvirus gB homologues (Fitzpatrick et al., 1990; Pereira, 1994). Although MAb
79/9 recognized the recombinant fragment encompassing aa 59–126 but not recombinant proteins that began at aa 86 (Fig. 1), in Fig. 3 we have not restricted its epitope to the region between aa 59 and 86 because it cannot be ruled out that some residues downstream of aa 86 are also involved in the formation of this epitope. The lack of reactivity of MAbs 86/15 and 13 with the recombinant protein that encompassed aa 59–126 may be attributed to the presence of GST attached to the N terminus of the recombinant fragment. Although the epitopes of MAbs 86/15 and 13 possess the properties of linear epitopes (Zaripov et al., 1998), it is not excluded that a particular local conformation of the polypeptide chain is required for their full expression; GST attached to the recombinant protein may affect the local conformation of the N-terminal part of the protein and, as a consequence, the expression of these epitopes.

The MAb 21/2 epitope is located in the segment between residues 214 and 279. Previously, we have shown that this epitope is probably an internal epitope of gB and is shielded in residues 214 and 279. Previously, we have shown that this expression of these epitopes. N-terminal part of the protein and, as a consequence, the recombinant protein may affect the local conformation of the particular local conformation of the polypeptide chain is required for their full expression; GST attached to the recombinant protein may affect the local conformation of the N-terminal part of the protein and, as a consequence, the expression of these epitopes.

The MAb 21/2 epitope is located in the segment between residues 214 and 279. Previously, we have shown that this epitope is probably an internal epitope of gB and is shielded in the native glycoprotein (Zaripov et al., 1998). Fragmentation of gB by CNBr and trypsin enhanced the reactivity of the proteins with MAb 21/2.

The residues involved in the assembly of the cluster of 10 discontinuous epitopes of PrV gB recognized by 22 MAbs were mapped to a region that includes all but the 38 N-terminal residues of the putative ectodomain of gBc, which is consistent with the data on epitope mapping of gB homologues from a number of herpesviruses (Fitzpatrick et al., 1990; Pereira, 1994). The constituents of two epitopes recognized by MAbs 24/11 and 34/2 were restricted to a segment encompassing aa 540–646. The residues that are implicated in the assembly of the remaining eight discontinuous epitopes were localized to a region between aa 540 and 734. Though 20 MAbs that recognize these eight epitopes did not react with the shorter fragment of gBc (aa 540–646), the constituents of the epitopes cannot be allocated to the segment between aa 647 and 734 because both regions of the ectodomain of gBc (540–646 and 647–734) may be necessary for the expression of these epitopes.

It should be noted that, on a Western blot, the reaction of MAbs specific for discontinuous epitopes of gB with non-reduced gB from virions was considerably stronger than that with reduced gB and recombinant fragments. This indicates that the discontinuous epitopes are only partially expressed in recombinant fragments. The residues of recombinant proteins that are recognized by the corresponding MAbs may represent the linear constituents of discontinuous epitopes. However, a partial restoration of discontinuous epitopes in recombinant proteins during Western blotting may also contribute to their reactivity with MAbs (Harper et al., 1990). The incomplete expression of these epitopes in E. coli-expressed recombinant proteins was probably due to the lack of authentic higher-order structure, which is inherent to prokaryotic expression systems (Cason, 1994). Also, it cannot be ruled out that these discontinuous epitopes include residues located outside the identified regions of gBc.

In this study, antibody-binding sites on the PrV gB molecule were mapped by using a previously characterized panel of 26 MAbs directed against gB, the specificity repertoire of which was shown to reflect adequately the epitope-specific antibody response to gB during the natural infection of swine (Zaripov et al., 1998). Our preliminary data strongly indicate that the antigenic map constructed with this panel does not differ substantially from the map obtained with sera from infected swine. For example, the N terminus of gB was also one of the major foci of anti-gB antibody response during the natural infection of swine (unpublished results).

In herpesviruses, the N terminus and the regions upstream of the transmembrane-spanning domain of gB were shown to harbour epitopes associated with neutralization (Fitzpatrick et al., 1990; Pereira, 1994). Previously, we also showed that the majority of continuous and discontinuous epitopes of PrV gB identified were associated with complement-dependent virus neutralization and induced a strong antibody response during natural infection of swine (Zaripov et al., 1998). Although no quantitative correlation has been reported between antibody level and clinical outcome, it seems likely that antibodies against immunodominant regions identified in this study could mediate the clearance of virus and virus-infected cells and contribute to protective immunity against PrV.

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References


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