Mapping of Antigenic Domains of Sendai Virus Nucleocapsid Protein Expressed in Escherichia coli

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Several nonoverlapping epitopes were mapped on the primary sequence of the Sendai virus NP protein. After a complete cDNA clone of the Sendai virus NP gene was expressed in Escherichia coli, deletion constructs were used to generate a series of overlapping NP fragments deleted at their C termini. Immunoblot analyses with 11 monoclonal antibodies identified four antigenic sites. All of these sites resided in the C-terminal half of NP and were also the only sites detected with a polyclonal serum. These findings confirm and extend the evidence that the C terminus of the NP protein represents the domain exposed on the surface of the nucleocapsid. One of the monoclonal antibodies reacted with a site, comprising only 6 amino acids, lying with a hinge between an α-helix and a β-strand in the predicted secondary structure of NP. Since this antibody is a potent inhibitor of in vitro viral RNA synthesis (K. L. Deshpande and A. Portner, Virology 139:32-42, 1984), the epitope may be critical to the flexibility of the NP molecule that makes the RNA template accessible during RNA synthesis.

Sendai virus, a useful laboratory model of the medically important paramyxovirus family, contains a ribonucleoprotein core (nucleocapsid) enclosed in a membrane derived from the host cell (4). The nucleocapsid serves as the template for viral RNA synthesis (11). The major structural component of the nucleocapsid is the NP protein, comprising 517 amino acids (10).

Previous studies showed that some monoclonal antibodies (MAbs) against NP protein inhibited virus-specific RNA synthesis (6). To relate the functional effects of these MAbs to the structure of the protein, we have now mapped their epitopes on deletion constructs made in bacteria.

To express NP protein in Escherichia coli, the NP gene was inserted in the expression plasmid pRC23 (5). Transcription of a heterologous gene inserted in pRC23 is driven by the λpL promoter which is under the control of the temperature-sensitive cI repressor encoded by the compatible plasmid pRK248cIts (1). Therefore, the expression of a heterologous gene is repressed during bacterial growth at 30°C and induced by shifting to 42°C. The strategy used in the construction of plasmids pRC-NP and pGT-NP for Sendai virus NP expression is shown in Fig. 1. In brief, the NP-coding sequence was placed between the ribosome-binding site of expression vector pRC23 and a synthetic translation terminator sequence. This sequence permitted the precise termination of protein fragments expressed from deletion constructs of pGT-NP.

The expression of NP protein by E. coli in the presence or absence of an active λ repressor was detected by immunoblotting with MAbs (Fig. 2, lanes 1 to 4). Bacterial cultures containing only pRC-NP or pGT-NP and grown at 37°C synthesized about 20 times more NP protein than cultures containing pGT-NP and the temperature-sensitive repressor after induction (data not shown). A rapid decline in NP protein synthesis on passage of bacteria lacking an active repressor indicated that NP expression was toxic. Therefore, all further work was done with bacteria containing the repressor-expressing plasmid pRK248cIts in addition to the NP expression vectors.

Bacterial expression of NP protein provided an opportunity to delineate the epitopes of a panel of MAbs by deletion mapping within the primary structure of the protein. A set of deletion constructs containing overlapping fragments of the NP gene was generated by deleting DNA fragments from the 3' end of the NP gene from expression plasmid pGT-NP (Fig. 3A). Constructs were confirmed by restriction analyses of the plasmid DNAs (2). Their ability to synthesize NP protein fragments truncated at their C termini was tested by in vitro coupled transcription-translation of isolated plasmid DNAs (Fig. 3B). The electrophoretic mobility of the largest polypeptide in each lane was in good agreement with its predicted size. Smaller bands were probably products initiated from AUG codons downstream of the first initiating AUG in the NP mRNA. A protein band with an M₅ of 34,000 was present in all cases, except the control. This band may represent the product of the Amp₅ gene encoded in the plasmid DNAs. Similarly, another light band which fortuitously migrated at the same rate as full-length NP protein was present in all samples and is therefore assumed to be a bacterial protein.

Of 16 MAbs tested, 11 reacted with the NP fragments (Fig. 4). The five MAbs which failed to react in the immunoblots had been selected by reaction with native NP in enzyme-linked immunosorbent assays (6) and presumably had noncontinuous epitopes which were destroyed by denaturation before electrophoresis. Two antibodies (M8 and M52) reacted only with full-length NP protein (Fig. 4A and B), showing that their epitopes were lost with deletion of the C-terminal 62 amino acids of NP which we have designated region I. Five antibodies (M13, M17, M19, M73, and WS16) reacted only with the second largest peptide and full-length NP (Fig. 4C and D), indicating that their epitope resides in a region of 30 amino acids between residues 425 and 455 (region II). This region contained three successive β-turns that have been predicted to reside in the secondary structure of the protein (10). Four other antibodies (M4, M6, M10, and

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of EcoRI-BamHI-digested the first 4G coincides cutting containing (region III).

Abbreviations: E3, phage promoter, and cloned first into M40; R, ampicillin-resistant. Symbols: E3, phage promoter, and cloned first into M40; E, E., T, TaqI site; B, BgIII site. Arrows indicate the direction of transcription of the NP gene.

FIG. 1. Construction of the NP expression plasmid. The NP cDNA cloned at the PstI site of plasmid pUC7 was isolated by cutting at the BamHI sites. The noncoding sequence between the 5' and the first AUG of the NP gene was deleted by ligation into EcoRI-BamHI-digested pUC9 of two complementary synthetic oligomers with EcoRI and TaqI cohesive ends (31 and 29 bases, respectively) together with a 1.6-kilobase TaqI-BamHI DNA fragment obtained by partial digestion of NP cDNA with TaqI. The plasmid DNA from an ampicillin-resistant clone was isolated and sequenced from its EcoRI site to verify the nucleotide sequence around the initiating AUG of the NP gene (3). To construct expression plasmid pGT-NP, the EcoRI-BamHI fragment containing the NP gene was isolated and cloned first into pRC23 and then, along with the p1, promoter, into pUC-T in E. coli RRI(ach57). Plasmid pUC-T was constructed by cloning a synthetic universal translation terminator (Pharmacia, Inc.) at the HindIII site of pUC9. All plasmids are ampicillin-resistant. Symbols: p, phage promoter p1, and the ribosome-binding site; —,—, universal translation terminator sequence. Abbreviations: B, BamHI site; T, TaqI site; E, EcoRI site; Bg, BgIII site. Arrows indicate the direction of transcription of the NP gene.

Research, Inc.) and NP protein electroeluted from a sodium dodecyl sulfate-polyacrylamide gel (9). Like the MAbs, anti-NP serum failed to detect the protein products of the deletion constructs d, e, f, and g (Fig. 4F). A significant finding was the reaction of all of the MAbs with the C-terminal half of the protein. Biochemical studies indicated that the C-terminal 120 amino acids of NP reside on the surface of the virus core (7). We have now located regions which lie on the surfaces of NP molecules within the viral nucleocapsids at least 100 more amino acids further towards the N terminus. Selection of the MAbs used in this work by enzyme-linked immunosorbent assay of binding to native nucleocapsids from virions may therefore explain the absence of any reactivities with the N-terminal half of the NP protein. It is more difficult to understand why the polyclonal serum failed to react with polypeptides representing this region. However, the isolation of MAbs restricted to limited regions of viral proteins has been reported for the NS protein of vesicular stomatitis virus (14), the P protein of Sendai virus (13), and the α4 protein of herpes simplex virus 1 (8). Perhaps all of these cases represent regional clustering of strongly antigenic sites, a possibility that may have implications for understanding immune defenses against viral infections.

The epitope for one of the antibodies (M4) was localized to a region of 6 amino acids (residues 290 to 295). An analysis of the predicted NP secondary structure (10) identified this region as a random coil between an α-helix and a β-strand. This portion of the NP molecule contains a proline residue and may serve as a hinge between the α-helix and the β-strand, conferring flexibility on the protein and the virus cores and therefore permitting the template RNA to be
FIG. 3. Deletion mapping of the NP gene and analyses of the protein products. (A) The NP gene is shown as a solid line with the restriction sites used for generating deletion constructs. Sites of initiation (↑) and termination (↓) of translation in the authentic protein are shown. To generate deletions in the NP gene, the plasmid pGT-NP was cut at the indicated restriction sites and at the 3' end of the gene at the BamHI site. Promining ends of DNAs were made blunt with the Klenow fragment of DNA polymerase I, and plasmids were recircularized with T4 DNA ligase and used for transformation of E. coli RR1(αcl857). The sizes of the protein products of deletion constructs are shown by bars and also given in numbers of amino acids (aa). (B) Radiolabeled polypeptides were synthesized in an in vitro procaryotic coupled transcription-translation system from plasmid DNAs (lanes a through h, deletion constructs shown in panel A) and analyzed on a sodium dodecyl sulfate-polyacrylamide gel (9). Products of a control reaction without any added plasmid DNA and 14C-labeled size markers are shown in lanes i and j, respectively. Protein products were detected by fluorography.

FIG. 4. Immunoblot analyses of the truncated NP polypeptides. Polypeptides from E. coli RR1(pRK248cIts) containing deletion constructs a through h (lanes a through h; constructs shown in Fig. 3A) were prepared as described in the legend to Fig. 2 after induction at 42°C for 3 h. Samples were separated by electrophoresis. Polypeptides were transferred to nitrocellulose, which was incubated with individual MAbs for immunoblot assay as described in the legend to Fig. 2. Immunoblots of antibodies M8, M52, WS16, M73, M6, polyclonal serum, M4, and M40 are shown in panels A through H, respectively.
exposed for interaction with the nucleocapsid-associated RNA polymerase during RNA synthesis. This may explain why binding of this particular antibody to NP in nucleocapsids strongly inhibits RNA synthesis in vitro (6).

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