Structural Effects of Amino Acid Substitutions on the Matrix Protein of Vesicular Stomatitis Virus

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The structural effects of amino acid substitutions for Gly at position 21 in the amino-terminal segment (Lys 15–Pro 26) of the matrix (M) protein of vesicular stomatitis virus (VSV) have been investigated using conformational energy analysis. Monoclonal antibody-binding experiments and protein digestion studies of the M protein indicate that this segment is important to its ribonucleoprotein recognition and its transcription-inhibitory activity. Temperature-sensitive mutants of VSV that do not bind monoclonal antibody and that are devoid of transcription-inhibitory activity are known to have the substitution of Glu for Gly at position 21. The current findings demonstrate a significant conformational change at position 21 induced by the substitution of Glu for Gly, which could explain this alteration in antibody binding and transcription-inhibitory activity. Furthermore, the results indicate that the substitution of any noncyclic L-amino acid for Gly at position 21 may be expected to produce similar changes in M protein function.

KEY WORDS: conformational energy; three-dimensional structure; amino acid substitutions; matrix protein; vesicular stomatitis virus.

INTRODUCTION

The matrix (M) protein of vesicular stomatitis virus (VSV), the prototype rhabdovirus, contains 229 amino acids (Rose and Gallione, 1981) and lines the inner surface of the lipid bilayer of the viral envelope in close association with the ribonucleoprotein (RNP) core (Newcomb et al., 1982; Zakowski and Wagner, 1980). The M protein appears to play an important regulatory role in virus-directed RNA synthesis in infected cells via its ability to inhibit transcription by the viral polymerase (Carroll and Wagner, 1979; Clinton et al., 1978). Recent monoclonal antibody

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experiments demonstrating reversal of the inhibition of viral transcription (Pal et al., 1985) in combination with M-protein digestion studies (Ogden et al., 1986) suggest that the RNP recognition site is somewhere in the region of amino acids 18–43. Furthermore, temperature-sensitive (ts) mutants in complementation group III of VSV-Indiana are known to have altered M protein genes (Pringle, 1977; Morita et al., 1987). A ts mutant derived from the Orsay wild-type (wt) strain, called ts023(III), contains M protein completely devoid of transcription-inhibitory activity and does not bind monoclonal antibody (Pal et al., 1985) directed to an antigenic determinant located between amino acids 18–43 (Ogden et al., 1986). Gene sequencing of the M-protein gene for ts023(III) reveals the substitution of a glutamic acid for the glycine of the wt protein at position 21 in the amino-terminal RNP recognition region of the M protein (Gopalakrishna and Lenard, 1985; Morita et al., 1987). The amino acid sequence for the first 30 residues of the M protein of the Orsay wt strain of the VSV-Indiana is (Gopalakrishna and Lenard, 1985):

<table>
<thead>
<tr>
<th>Met-Ser-Ser-Leu-Lys-Lys-Ile-Leu-Gly-Leu-10</th>
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<tbody>
<tr>
<td>Gly-Ile-Ala-Pro-Pro-Pro-Tyr-Glu-Glu-Asp-30,</td>
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where the critical residue 21 is italicized. The importance of the amino acid in this position for maintaining biological integrity was further characterized by generating synthetic peptides corresponding to the amino acid sequence from position 17–31 of both the wild-type and mutant proteins. The oligopeptide corresponding to the wild-type sequence was recognized by monoclonal antibody directed to the same region of the wild-type protein, and the oligopeptide was able to compete with wild-type M protein for monoclonal antibody binding (Shipley et al., 1987). In this study, it was further shown that the oligopeptide corresponding to the wild-type sequence gave rise to antibodies in rabbits that not only bound to the wild-type protein but were also able to reverse the transcription inhibitory activity as well. The oligopeptide corresponding to the mutant protein sequence was not recognized by the monoclonal antibody, and did not give rise to antibody which recognized wild-type M protein. These data suggest that a single amino acid substitution at position 21 of the M protein gives rise to an altered surface topology that is not recognized by antibody to the wild-type protein and that is deficient in transcription-inhibitory activity.

These considerations raise the possibility that the amino acid substitution at position 21 of the M protein causes a significant structural change in the protein that accounts for its altered transcription-inhibitory activity and that may be responsible for the diminished affinity the substituted protein exhibits for the monoclonal antibody. Therefore, in this paper, we have examined the structural effects of amino acid substitutions at position 21 of the VSV M protein using conformational analysis to determine the preferred conformations for the dodecapeptide Lys 15–Pro 26 containing glycine, glutamic acid, alanine, and proline at position 21.

METHODS

The general methods used are based on the Empirical Conformational Energies of Polypeptides and Proteins (ECEPP) program (Momany et al., 1975; Nemethy