Identification of viral mutants by mass spectrometry

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ABSTRACT A method to identify mutations of virus proteins by using protein mass mapping is described. Comparative mass mapping was applied to a structural protein of the human rhinovirus Cys119 → Tyr mutant and to genetically engineered mutants of tobacco mosaic virus. The information generated from this approach can rapidly identify the peptide or protein containing the mutation and, in cases when nucleic acid sequencing is required, significantly narrows the region of the genome that must be sequenced. Accurate mass measurements and tandem mass spectrometry can then be used to definitively identify the amino acid substitution; a schematic of this approach is shown in Fig. 1.

MATERIALS AND METHODS

Tobacco Mosaic Virus (TMV) Constructs. The known atomic structure (5) of TMV was used to select two amino acids in the coat protein (CP) for mutation. Glu-50 and Asp-77 are located on the right sleeked (RS) and right radial (RR) α-helices of the CP, respectively. The CP mutants Glu-50 → Gln, Glu-50 → Met, Asp-77 → Asn, and Asp-77 → Arg (M.B. and R.N.B., unpublished work) were generated by PCR-based site-directed mutagenesis using the plasmid pKN2 (6) containing the full-length TMV CP. The mutant CP genes were then used to replace their homologue in the TMV cDNA clone U3/12-4 (7) to generate infectious full-length cDNA clones: pTMV-Glu-50Gln, pTMV-Glu50Met, pTMV-Asp77Asn, and pTMV-Asp77Arg.

Purification of Virus Particles from Infected Plants. Full-length transcripts were produced with T7 RNA polymerase from the wild-type pTMV (U3/12-4) and mutant pTMV cDNA clones as described previously (7) and used to inoculate 4-week-old Nicotiana tabacum Linnaceae cv. Xanthi nn plants. Ten to 15 days after inoculation, virus particles were purified from systemically infected leaves as described previously (8). Infected leaf material was ground in liquid N2 and homogenized in the extraction buffer (0.5 M Na2HPO4/0.5% sodium ascorbate). Cellular debris was removed by centrifugation and chloroform was removed by extraction with diatomaceous earth (grade III, Sigma). Virus particles were precipitated twice in the presence of 3% PEG8000 and 1% NaCl, washed with 5% Triton X-100, and collected by centrifugation at 90,000 × g. Virus particles were further washed (two times) with 200 mM sodium phosphate, pH 7, for 4 hr at 37°C and collected by centrifugation at 90,000 × g. The pure virus particles were then resuspended in water or in 10 mM Tris-HCl, pH 7.2/1 mM EDTA to a final concentration of 1 mg/ml and stored at 4°C. Mutant virus particles were indistinguishable from wild-type virus particles by electron microscopy (data not shown).

Production of Mutant Human Rhinovirus 14 (HRV14). The HRV14 mutant (Cys-199 → Tyr in structural protein 1), a naturally occurring spontaneous mutant of HRV14, was selected for by isolating plaques that developed when wild-type

Abbreviations: MALDI, matrix-assisted laser desorption/ionization; TMV, tobacco mosaic virus; HRV14, human rhinovirus 14; FTMS, Fourier transform mass spectrometry; TOF, time-of-flight; MS, tandem mass spectrometry.

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