Detection of a Genome-Linked Protein (VPg) of Hepatitis A Virus and Its Comparison with Other Picornaviral VPgs

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The nucleotide sequence corresponding to the P3 region of the hepatitis A virus (HAV) polyprotein genome was determined from cloned cDNA and translated into an amino acid sequence. Comparison of the amino acid sequences of the genome-linked proteins (VPg) of other picornaviruses with the predicted amino acid sequence of HAV was used to locate the primary structure of a putative VPg within the genome of HAV. The sequence of HAV VPg, like those of other picornaviral VPg molecules, contains a tyrosine residue as a potential binding site for HAV RNA in position 3 from its N terminus. The potential cleavage sites to generate VPg from a putative HAV polyprotein are between glutamic acid and glycine at the N terminus and glutamic acid and serine or glutamine and serine at the C terminus. A synthetic peptide corresponding to 10 amino acids of the predicted C terminus of HAV VPg induced anti-peptide antibodies in rabbits when it was conjugated to thyroglobulin as a carrier. These antibodies were specific for the peptide and precipitated VPg, linked to HAV RNA, from purified HAV and from lysates of HAV-infected cells. The precipitation reaction was blocked by the synthetic peptide (free in solution or coupled to carrier proteins) and prevented by pretreatment of VPg RNA with protease. Thus, our predicted amino acid sequence is colinear with the nucleotide sequence of the VPg gene in the HAV genome. From our results we concluded that HAV has the typical organization of picornavirus genes in this part of its genome. Similarity among hydrophobicity patterns of amino acid sequences of different picornaviral VPgs was revealed in hydropathy plots. Thus, the VPg of HAV appears to be closely related to VPg1 and VPg2 of foot-and-mouth disease virus. In contrast, HAV VPg has a unique isoelectric point (pI = 7.15) among the picornavirus VPgs.

Hepatitis A virus (HAV) has been classified on the basis of its physical, biochemical, and morphological features as a member of the family *Picornaviridae* (16). Results obtained from the molecular cloning and characterization of its complete genome indicated that its organization resembles that of other picornaviruses (8, 23, 30, 45). In addition, the genomic location of capsid protein genes was assessed by determination of the amino acid sequence of the N termini of VP1 and VP3 (23). Based on surface probability analyses of its presumed amino acid sequence, a peptide corresponding to part of VP1 was selected and synthesized (12). This peptide induced neutralizing antibodies to HAV, thus corroborating the results of Linemeyer et al. (23).

The basic genomic structure of the picornaviruses was largely derived from data on poliovirus (PV) type 1 (20, 34, 35). The genes within the positive-stranded picornaviral RNA were grouped into three regions: P1, P2, and P3 (see Fig. 1). The P1 region is preceded by a noncoding 5′ sequence of about 700 to 1,200 nucleotides, and the P3 region is followed by a short noncoding sequence and a poly(A) 3′ terminus. P1 comprises the capsid proteins (VP1 to VP4), and P2 contains nonstructural proteins which have not been well characterized. The P3 region codes for the replicase, a protease, and a small protein (Mr ≈ 2,500; 20 to 24 amino acids) called VPg for genome-linked viral protein. VPg is a component of the mature virus particle (47).

VPg is covalently attached to the 5′ end of viral RNA (13, 22) through a phosphodiester bond between tyrosine and the terminal uridylyl residue of the RNA to form the following structure: VPg(Tyr-O')pUpU... (2, 37). In infected cells, VPg is present on viral RNA plus and minus strands, on the double-stranded replicative form, and on nascent plus strands of PV replicative intermediates (13, 23, 31, 33, 48). VPg was also detected in the unbound form and as VPg-pUpU in PV-infected cells (11).

From these findings and studies of PV RNA replication in vitro, it was concluded that VPg, or precursors thereof, are involved in the replication of viral RNA and, as nucleotidyl derivatives, might serve as primers in the synthesis of viral RNA (7, 29, 44). However, the role of VPg in RNA replication remains unsettled (3). Involvement of picornaviral VPgs in the synthesis of viral proteins has been excluded because viral mRNA has no VPg bound to its 5′ end (32). It has also been suggested that the protein is involved in the morphogenesis of progeny virus (5, 19). Whatever its function, VPg is conserved among all genera of the *Picornaviridae* (reviewed in reference 47), suggesting an essential role of this protein in the viral life cycle.

Although knowledge of the properties of HAV was sufficient for its taxonomic classification, information about the HAV genes that are actually expressed during viral replication is limited (cf. reference 16). This is particularly true for the nonstructural proteins of the virus and is in part due to difficulties in studying HAV because of its slow and inefficient replication in vitro (41). Furthermore, metabolic labeling of viral proteins by radioisotopic precursors is greatly hampered because cellular protein synthesis is not inhibited by the virus (24). To overcome this disadvantage in the detection of a VPg in HAV, we combined two basic approaches: immunoprecipitation of VPg with antisera against

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a synthetic peptide, the amino acid sequence of which was predicted from the nucleotide sequence of cloned HAV cDNA, and use of cloned HAV cDNA to detect, by hybridization, immunoprecipitated macromolecules of VPg linked to viral RNA. This approach enabled us to demonstrate the presence of the genome-linked VPg in purified HAV and cells infected with the virus.

**MATERIALS AND METHODS**

**Virus and cells.** Uninfected BSC-1 cells and BSC-1 cells that were persistently infected with HAV strain HM175 (S. M. Feinstone, personal communication) were grown in Eagle minimal essential medium containing 10% fetal bovine serum. HAV-infected cells were maintained for 8 to 12 days in roller bottles. For propagation of PV type 1, Sabin. cells were infected with virus and harvested when the cytopathic effect was complete, usually within 3 to 4 days. Both PV and HAV were isolated and purified as described previously (46) except that poliovirus was concentrated only from the supernatant medium.

**Peptide synthesis and preparation of antiserum.** The peptide was prepared by the solid-phase method of peptide synthesis (26) with a Beckman 990 peptide synthesizer. A cysteine residue was added to the N terminus to facilitate coupling to the carrier protein, and a tyrosine residue was added to the C terminus to facilitate radiolabeling. The synthetic peptide was cleaved from the solid support with liquid HF and purified by gel filtration chromatography. The purity of the peptide was verified by amino acid analysis. The purified peptide was coupled to thyroglobulin and bovine serum albumin (BSA), respectively, through the free sulphydryl group with the bifunctional cross-linking agent n-maleimidobenzoyl-N-hydroxysuccinimide (15). The peptide-thyroglobulin conjugate was mixed with Freund complete adjuvant, and 200 µg was injected into each of two rabbits. Subsequent immunizations were at 2, 3, and 13 weeks with 200 µg of conjugate in Freund incomplete adjuvant. The rabbits were bled 4 and 15 weeks after the first immunization. A synthetic peptide (1A) and corresponding antiserum (α-1A) related to a murine H-2 class II antigen were used in control experiments.

**Immunoprecipitation and analysis of the antigen.** The cytoplasm of 10⁶ cells was prepared as described by Maniatis et al. (25) except that aprotinin (Sigma) was added to the cell lysates to a final concentration of 0.1% (wt/vol) and extraction of proteins was not routinely performed. Sodium dodecyl sulfate and mercaptoethanol were added to final concentrations of 2% (wt/vol) and 5% (vol/vol), respectively, and the solutions were heated at 100°C for 3 min and immediately transferred to an ice water bath. After dilution to 12 times the original volume of 2.87 ml in TEN (50 mM Tris hydrochloride [pH 7.6], 140 mM NaCl, 5 mM EDTA, 0.5% [vol/vol] Nonidet P-40), RNAsin (Promega Biotech) and aprotinin were added to restore their original concentrations. Denaturation of approximately 10⁷ CHO cells culture infusives doses of purified HAV or PV (measured by immunofluorescence and cytopathic effects, respectively) was similarly achieved.

Subsequent experiments were performed with 200-µl samples of denatured cell or virus lysates equivalent to approximately 10⁶ cells and 10² viruses, respectively, that had been preadsorbed with 50 µl of sedimentsed protein A-Sepharose CL-4B (Pharmacia) to remove nonspecifically adhering components. Protein A-Sepharose CL-4B was routinely sedimented in a microcentrifuge at 12,000 x g for 1 min.

For immunoprecipitation, 10 µl of undiluted serum was added to lysates. Immune complexes were allowed to form at room temperature for 2 h and were precipitated with 60 µl of protein A-Sepharose (50% [vol/vol]) at room temperature for 30 min, followed by centrifugation at 12,000 x g for 1 min in a microcentrifuge. The precipitated immune complexes were washed three times in 400 µl of TEN by centrifugation and suspension. Desorption of the antigen from protein A-Sepharose was achieved by incubation in 50 µl of 0.1 M glycine/hydrochloride (pH 3.0)–0.5% Nonidet NP-40 (vol/vol) at room temperature for 10 min, followed by centrifugation. The protein A-Sepharose was then washed in 25 µl of the same buffer. The supernatants of the elution and the washing step were combined and diluted into 400 µl of 25 mM sodium phosphate buffer (pH 7.0) and filtered onto a nylon membrane (Zeta- Probe: Bio-rad Laboratories) in a manifold apparatus (Minifold II; Schleicher & Schell, Inc.). The nylon membrane was subsequently air dried and baked in vacuo at 80°C for 2 h. Hybridization of [³²P]-labeled cloned HAV cDNA (45) was performed as described by Church and Gilbert (10). Cloned PV type 1 cDNA for detection of PV RNA by hybridization was a gift of G. Stanway and J. W. Almond (University of Leicester, United Kingdom).

Proteolysis of cytoplasmic proteins with proteinase K, extraction of proteins, and concentration of nucleic acids in ethanol were performed as described by Maniatis et al. (25). One unit of RNase-free DNase (RQ1; Promega) was incubated with immunoprecipitates at 37°C for 30 min in 100 mM sodium phosphate (pH 7.2)–10 mM MgCl₂–5 mM dithiothreitol to remove all DNA. RNA was hydrolyzed in 0.3 M NaOH for 30 min at room temperature. Controls of the hydrolyses were performed with Escherichia coli rRNA and salmon sperm DNA under identical conditions.

The proteins and peptides used to inhibit immunoprecipitations were dissolved in H₂O at a concentration of 64 mg/ml and incubated with 10 µl of antiserum prior to incubation with lysates at the ratios indicated in the figure legends. These incubations were at room temperature for 2 h with 1 U of RNasin per µl-1% (wt/vol) aprotinin.

**Analysis and comparison of sequences.** Nucleotide sequences of purified cloned HAV strain HM-175 cDNA fragments were determined (27), and an amino acid sequence of the P3 region was predicted. The putative HAV VPg amino acid sequence was located by visual comparison with VPg amino acid sequences of other picornaviruses (4) and by use of the SEQHP and SEQDP programs (18) in a VAX 11/700 computer.

**RESULTS**

Nucleotide sequence analysis of plasmids pHAV₁₉₂₄ and pHAV₁₉₁₀₈ (45) resulted in a sequence of 2,925 bases from the 3' end of the HAV genome and extended the published sequence (8; unpublished data). Translation of this sequence into amino acid sequences revealed a continuous open reading frame (only shown for VPg in Fig. 1).

To locate the amino acid sequence of a VPg visually in the HAV polyprotein, an outstanding feature of other picornavirus sequences, for VPg was used as a probe against which VP RNA is covalently linked (13, 22), is the third residue from the N terminus in VPgs of PV and other picornaviruses. In HAV we located a putative N-terminal glutamic acid-glycine cleavage site for a protein with a tyrosine residue in position 3, which corresponds to the configuration in foot-and-mouth disease virus (FMDV) VPgs 1, 2, and 3 (14). We predicted an amino acid sequence of 21 or 23 amino acids.
depending on whether the C terminus of HAV VPg is generated by cleavage between glutamic acid and serine or between glutamine and serine. The former corresponds to the C-terminal cleavage site in FMDV VPg3.

None of the features outlined to characterize a picornaviral VPg applied to the proposed HAV VPg sequence previously reported (30). We therefore performed a search for homology of our putative P3 amino acid sequence with those of other picornaviruses. With the SEQHP and SEQDP programs (18), homology was detected between the amino acid sequences of HAV and FMDV type A12 (36) when the complete P3 regions were compared. The parameters of homology determined by SEQDP were −318 for the distance; the significance in standard deviation units was 19.38 based on distances of 20 randomized sequences. No amino acid sequence with significant homology to HAV was found when only the putative sequence of the 3AB portion of P3 was used. A visual alignment, based in part on the result from SEQHP, of HAV and FMDV VPgs with that of PV type 1 is shown in Fig. 1.

To determine whether the putative sequence actually represented HAV VPg, a peptide (CVPg) was synthesized corresponding to the 10 C-terminal amino acids (Fig. 1). For labeling purposes, a tyrosine residue was added at the C terminus; a cysteine residue at the N terminus served to couple the peptide to carrier proteins. Rabbits inoculated with CVPg coupled to thyroglobulin developed an antibody response to the synthetic peptide that was detected by radioimmunoassay (data not shown). One antiserum (α-CVPg) was used to attempt precipitation of VPg from HAV-infected cells and from purified virus (Fig. 2). Immunoprecipitates were formed that presumably contained HAV RNA because they were detected by hybridization with cloned HAV cDNA (Fig. 2, lines A and C). No precipitation was detected when uninfected cells were tested with α-CVPg (Fig. 2, line B) or when TNEN buffer was used instead of antisem (Fig. 2, line E). The preimmune serum (pre-CVPg) of the rabbit showed a background reactivity with infected cells (Fig. 2, line D). This reaction was not specific for CVPg, because addition of the synthetic peptide to pre-CVPg before incubation with lysate did not reduce the amount of antigen precipitated (Fig. 2, line F).

To show that precipitation of HAV RNA was mediated by VPg, we assessed the immunochromosomal specificity of α-CVPg. Specificity for CVPg was shown by inhibition of immunoprecipitation of VPg by CVPg and by failure of unrelated antigens to block the immunoprecipitation from infected cells (Fig. 3). CVPg, either alone (Fig. 3, line A) or coupled to carrier protein BSA (Fig. 3, line B) and thyroglobulin (data not shown), reduced the immunoprecipitation reaction of α-CVPg to background level in a concentration-dependent manner. Neither the carrier proteins thyroglobulin (data not shown) or BSA alone (Fig. 3, line C) nor peptide IA (unrelated to CVPg) blocked the reaction (Fig. 3, line D). In addition, a rabbit antiserum directed against the unrelated peptide (α-IA) did not precipitate VPg RNA from cells infected with HAV (Fig. 3, line E).

To test whether HAV VPg shared any immunochromosomal crossreactivity with VPg of PV, we purified and denatured PV type 1, Sabin, and tried to immunoprecipitate its RNA with α-CVPg under standard conditions (data not shown). No PV RNA was detectable by hybridization with cloned PV cDNA after this treatment. In contrast, a control sample of the viral lysate before attempted immunoprecipitation contained detectable PV RNA.

To exclude the possibility that the HAV-specific macromolecules were precipitated and detected by trapping in complex mixtures and to confirm that α-CVPg reacted with a protein bound to HAV RNA and not with the nucleic acid itself, we characterized the immunoprecipitate. Lysates of HAV and HAV-infected cells were degraded with proteinase K, extracted, and concentrated. After this treatment, no detectable antigen was precipitated by α-CVPg (Fig. 4, no. 3), although HAV RNA was abundant in the treated lysates (Fig. 4, no. 1 and 2). In contrast, neither extraction and

![Diagram](https://example.com/diagram.png)

**FIG. 1.** Structure of the picornavirus genome, proposed location of HAV VPg, and amino acid sequences. (A) The major regions of the picornavirus polypeptide encoded by the viral RNA. The approximate end products of proteolytic cleavage of the polypeptide are also shown. (B) The location of VPg on the viral genome and a visually derived alignment of the amino acid sequences of different VPgs with our predicted HAV VPg sequence: PV type 1. Sabin (20); FMDV type A12 (36; see the text). The amino acid sequence of the synthetic peptide CVPg is also included. The nomenclature is as described by Rueckert and Wimmer (38). Slashes indicate putative cleavage sites. kb, Kilobases.

![Diagram](https://example.com/diagram.png)

**FIG. 2.** Immunoprecipitation of VPg RNA from purified HAV and HAV-infected BSC-1 cells. Immunoprecipitations were performed with 200 μl of lysates and 10 μl of antiserum or TNEN buffer. (A) Purified HAV particles and α-CVPg; (B) cytoplasm of uninfected BSC-1 cells and α-CVPg; (C) cytoplasm of HAV-infected BSC-1 cells and α-CVPg; (D) cytoplasm as in C and pre-CVPg; (E) cytoplasm as in C and TNEN buffer only; (F) cytoplasm as in C and pre-CVPg, preincubated with 170 μg of synthetic peptide CVPg. All immunoprecipitates were filtered onto a nylon membrane and detected after hybridization with 32P-labeled cloned HAV cDNA by autoradiographic exposure (16 at −70°C with two intensifying screens).
concentration nor proteinase K digestion affected detection of previously formed immunoprecipitates (Fig. 4, A, B, and C). To demonstrate that the molecular species detected in immunoprecipitates from HAV-infected cells was HAV RNA, hydrolysis of nucleic acids was performed with alkali or DNase. Degradation of immunoprecipitates by 0.3 M NaOH (Fig. 4, E), but not by RNase-free DNase (Fig. 4, F), showed that the protein-linked nucleic acid was RNA.

To compare the amino acid sequence predicted for HAV VPg to VPgs of other viruses, hydropathy plots were made as described by Kyte and Doolittle (21) (Fig. 5B) and Hopp and Woods (17) (Fig. 5A). Both analyses revealed a high degree of similarity among the hydropathy profiles of PV, HAV, and FMDV VPgs. The profile of HAV VPg was very closely related to that of PV in the N-terminal half of the sequences when assessed as described by Kyte and Doolittle, but in both analyses HAV VPg appeared more closely related to VPg1 and VPg2 of FMDV when the entire profiles were visually compared.

When the hydropathies of individual amino acid residues were plotted, a striking similarity was revealed for the entire length of the sequences of FMDV VPg1 and VPg2 and HAV VPg (data not shown). In particular, an alternation of charged or hydrophilic residues with nonpolar residues appeared as an outstanding feature (cf. Fig. 1). This periodicity was less apparent in the VPg of PV. Comparison of the distribution of charges revealed a cluster in each half of the sequences. However, the polarity of the charges varied in the respective positions of the different sequences. These differences in composition of charged residues led to different predicted isoelectric points of the VPgs: HAV, pl = 7.15; PV1, pl = 11.4, and FMDV A12 VPg1, pl = 10.02 (calculated by using the DNA-Star program with the reference pH set to 7).

**DISCUSSION**

Short synthetic peptides have been successfully used as immunogens to elicit antibodies with a predetermined specificity (reviewed in references 1 and 43). To probe for a VPg in HAV, we synthesized a peptide (CVPg) that elicited an antibody response in rabbits to the VPg of HAV. A similar approach had been applied previously to detect PV-specific
polypeptides (6, 40). Although the rabbit preimmune serum contained nonspecific reactivities, we showed by inhibition tests that the hyperimmune serum (α-CVPg) specifically precipitated VPG RNA. Our experiments with protease K showed that VPG precipitation of HAV RNA by α-CVPg was due to a protein linked to the nucleic acid and not to interaction with the nucleic acid itself. Carrier proteins such as BSA and thyroglobulin and a synthetic peptide (IA) unrelated to CVPg had no influence on the precipitation reaction. In contrast, CVPg did block immunoprecipitation by α-CVPg, and this inhibition was most efficient when CVPg was coupled to carrier proteins. The reaction was completely blocked with coupled peptide that corresponded to 2% of the amount used in experiments with the free peptide. This suggested that conformations of the conjugated peptide more closely resembled the three-dimensional structure of the denatured VPG bound to HAV RNA than did conformations of the free peptide in solution. Alternatively, peptide interaction might have contributed to epitopes that were more readily recognized by the antisera than the free peptide alone. However, the free peptide did mimic the antigenic structure of VPG: whether this was on the basis of a mobility mechanism or a limited number of conformations (reviewed in reference 9) is not known. The specificity of α-CVPg thus revealed was further confirmed by the use of a rabbit anti-IA antisera, which did not precipitate VPG RNA of HAV. Furthermore, PV VPG RNA was not recognized by α-CVPg. The reactivity of α-CVPg in our experiments was thus confined to the predetermined target molecule, VPG of HAV, the presence of which was revealed by detecting the attached RNA. The results demonstrated linearity of the predicted amino acid sequence and the HAV gene coding for VPG. In addition, although the VPGs of the picornaviruses might be functionally related, the VPGs of PV type 1 and HAV share no common antigenicity of their C-terminal amino acid sequences.

To enable direct demonstration of HAV VPG, e.g., by gel electrophoresis as had been possible for PV VPG and its precursors (6, 40), the replication efficiency of HAV in tissue culture will have to be greatly enhanced. At present, only capsid proteins of HAV have been unambiguously identified. Detection of VPG is more difficult because there is only one such molecule as opposed to 60 copies of each capsid protein per picornavirus particle (cf. reference 34).

Our attempts to use the viral RNA as an intrinsic label for a genome-linked protein proved to be successful. Preparation of lysates by boiling them in the presence of strong denaturing agents or by extraction with protein-denaturing organic solvents did not remove the genome-linked protein from HAV RNA. Such treatments, which would normally disrupt noncovalent protein-nucleic acid interactions, were also unable to remove VPG from PV RNA (13, 22). This and the presence of a tyrosine residue in the HAV VPG sequence suggest that a covalent linkage of VPG to RNA exists. However, the covalent linkage has actually been demonstrated only for PV and FMDV (2, 37, 39). By analogy with all other picornavirus VPGs studied, the tyrosine residue of HAV VPG is the most likely site for attachment of the RNA, but we cannot strictly rule out a serine residue, perhaps at the second serine residue (40), as the acceptor amino acid. HAV VPG may be a mosaic virus, a plant virus that has many features that are homologous with picornaviruses (4), a serine residue has been claimed to bind the viral RNA (49). Serine residues of genome-linked proteins have also been shown to bind the 5′ ends of other viral genomes (47).

Direct experimental evidence that the RNA of HAV has the genomic organization of other picornaviruses has not been presented except for the capsid proteins (12, 23). Nevertheless, analysis of HAV nucleotide sequences by computer programs and deduction by analogy have permitted assignment of a putative order of the HAV genome (8, 30, 44a). The detection of a VPG demonstrates another feature of HAV that is found in picornaviruses and is consistent with HAV, a picornavirus genome organization for HAV in part of the P3 region.

Our results also indicate that the location of the VPG gene in the HAV genome predicted earlier by Najarian et al. (30) is unlikely to be correct. The latter reported VPG as a 23-amino-acid peptide with its N terminus generated by cleavage between histidine and phenylalanine and the C terminus generated by cleavage between glutamine and valine. In addition, although the proposed amino acid sequence included the tyrosine that we describe in position 3 from the putative N terminus, it was located in position 15 from a phenylalanine N terminus. In addition, this VPG did not contain the 10 C-terminal amino acids of our deduced sequence.

To assess the relationship of HAV VPG to those of other picornaviruses, we performed comparisons of the hydropaths of VPG sequences by using two scales (17, 21) which had been related to crystallographic and antigenic features of proteins. Although the two scales vary in the assignment of hydropathy values, particularly with respect to those for residues that are not clearly hydrophobic or hydrophilic, both analyses showed similarities among the VPGs of HAV, PV, and FMDV. Whether the similarities in the profiles of the plots contribute to a similar secondary or tertiary structure remains to be shown.

From our results, the HAV VPG amino acid sequence appears more closely related to VPG1 and VPG2 of FMDV, an aphthovirus, than to that of PV, an enterovirus (Fig. 5). This finding was corroborated by the results of our analysis of a much longer amino acid region using the homology programs of Kanehisa (18). HAV has been classified as enterovirus 72 (28), and one might have expected more similarity between the VPGs of PV and HAV. However, HAV has a number of unique features among the picornaviruses. In particular, nucleotide sequence homology with other picornaviruses was not detectable by computer analysis and standard procedures of molecular hybridization (J. R. Ticehurst, S. M. Feinstone, T. Chestnut, N. C. Tassopoulos, and R. H. Purcell; submitted for publication). The similarity between VPG amino acid sequences of HAV and FMDV also does not necessarily reflect similarities between other proteins of the respective genomes because the overall amino acid homology was more extensive between PV and FMDV than between HAV and either of the other two (8; J. Cohen, J. R. Ticehurst, and R. H. Purcell; submitted for publication). The degree of homology of proteins of two picornaviruses also depends on the particular protein investigated. A good example of this was established by comparison of human rhinovirus strains 2 and 14 with PV strain 1 (42).

Many functions for the VPGs in picornaviruses have been proposed, but these remain unproven (cf. references 5 and 47) assignment of dipeptide sequence VPG to the VPg amino acid residues in the N-terminal portion of HAV VPG appear to be conserved among other picornaviruses (4). Whether this and the similarities revealed in our analysis are related to a common function of the VPGs remains to be elucidated. Also, our calculated isoelectric point for HAV VPG distinguishes it markedly from the other VPGs of picornaviruses. This suggests that, if the VPGs are
involved in a common function, they may not accomplish it interchangeably.

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LITERATURE CITED