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A Single Antigenomic Open Reading Frame of the Hepatitis Delta Virus Encodes the Epitope(s) of Both Hepatitis Delta Antigen Polypeptides p24$^{\delta}$ and p27$^{\delta}$

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On the basis of the complete nucleotide sequence of the single-stranded, covalently closed circular hepatitis delta virus RNA genome (K.-S. Wang, Q.-L. Choo, A. J. Weiner, J.-H. Ou, R. C. Najarian, R. M. Thayer, G. T. Mullenbach, K. J. Denniston, J. L. Gerin, and M. Houghton, Nature [London] 323:508–514, 1986 [Author's correction, 328:456, 1987]), five long open reading frames (ORFs) encoding polypeptides containing a methionine proximal to the amino terminus were expressed in bacteria. Only polypeptides encoded by the antigenomic ORF5 cross-reacted with antisera obtained from patients with hepatitis delta virus infections. Immunological analysis of viral extracts and the recombinant ORF5 polypeptides synthesized in bacteria and yeast cells revealed that ORF5 encodes the immunogenic epitope(s) shared by both hepatitis delta viral polypeptides p27$^{\delta}$ and p24$^{\delta}$ and probably represents the complete structural gene for p27$^{\delta}$ and p24$^{\delta}$. We also present evidence that ORF5 encodes the hepatitis delta antigen, an antigen originally found in the nuclei of hepatocytes of infected individuals (M. Rizzetto, M. G. Canese, S. Arico, O. Crivelli, F. Bonino, C. G. Trepo, and G. Verme, Gut 18:997–1003, 1977). A comparison of the primary structure of the predicted hepatitis delta antigen polypeptides with that of the core antigen of the hepatitis B virus shows that these polypeptides are very dissimilar.

The hepatitis delta virus (HDV) is an infectious agent which requires an hepadnavirus helper function(s) for propagation in chimpanzee, human, or woodchuck hepatocytes (20, 24, 25). Similar in many respects to several viroidlike plant viral satellites (6, 13), the HDV genome is weakly related to the genome of the virus which provides these helper functions (31, 32). Individuals coinfected with HDV and hepatitis B virus or hepatitis B virus carriers superinfected with HDV frequently suffer severe forms of hepatitis which may result in fulminant hepatitis (27). Liver disease associated with HDV infections has been reported in several countries, including the United States (21, 26, 27), and is endemic in the Italian and north Colombian hepatitis B virus carrier populations (17, 22, 26). Groups at high risk for infection include hemophiliacs and intravenous drug users as a result of transmission of the virus through blood or blood products (22).

Purified 36-nm HDV particles contain an RNA genome (1,679 nucleotides) (3–5, 14, 25, 31) and HDV-specific polypeptides which are encapsidated by hepatitis B surface antigen and lipid (2, 3, 25). Treatment of HDV particles with detergents exposes an internal antigen which can be detected by sera from patients with HDV infections in radioimmunoassays and which could correspond with the nuclear hepatitis delta antigen (HDAg) (25). Recent immunoblot analysis of partially purified HDV particles revealed two polypeptides, p24 and p27, which specifically bind to sera from patients with chronic HDV infections (1, 2). The putative viral p27 and p24 polypeptides (referred to as P29^{d} and P27^{d} by Bonino et al. [2]) will be referred to here as p27$^{\delta}$ and p24$^{\delta}$. In this report, we establish that the HDV antigenomic open reading frame 5 (ORF5) encodes the epitope(s) of both polypeptides p27$^{\delta}$ and p24$^{\delta}$ which together compose the HDAg.

MATERIALS AND METHODS

Plasmid constructions and expression in bacteria. The following HDV cDNA fragments were excised from clones described in reference 31 and subcloned into the human superoxide dismutase expression plasmid (phSOD) described in reference 28 to generate the hSOD-ORF1, -2, -6, and -7 expression plasmids used in this report: (i) the δ1 *Nco*I 0.436-kilobase (kb) fragment; (ii) δ115, digested with *Bst*XI, followed by treatment with *Escherichia coli* DNA polymerase I large fragment (Klenow) enzyme and subsequent digestion with *Eco*RI to generate a 0.593-kb fragment; (iii) a δ4 *Sma*I-*Eco*RI 0.625-kb fragment; and (iv) a δ115 *Alu*I-*Sma*I 0.439-kb fragment, respectively. The ORF5 DNA sequence used in both yeast and bacterial expression vectors to generate the ORF5 polypeptide was derived from a δ115 *Sst*II-*Sal*I 0.605-kb DNA fragment ligated to a synthetic DNA linker which encoded the 10 putative *N*-terminal amino acids, including the predicted initiator methionine. Yeast expression plasmids will be described elsewhere (manuscript in preparation). All plasmids were transformed into *E. coli* RR1 ΔM15 (18, 19). Bacterial cultures (optical density at 650 nm = 0.6) harboring vector or recombinant expression plasmids were induced with 2 mM isopropyl-β-D-thiogalactopyranoside (IPTG) for approximately 4 h before the cells were pelleted and lysed in sodium dodecyl sulfate gel sample buffer (16).

Partial purification of ORF5 polypeptides from bacteria and yeast. One hundred milliliters of a bacterial culture (optical density at 650 nm ~ 0.6) expressing ORF5 or a control

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plasmid lacking HDV sequences was pelleted and suspended in 5.0 ml of buffer I (50 mM Tris hydrochloride [pH 8.0], 1 mM EDTA, 1 mM phenylmethyl sulfonyl fluoride, 1 μg of pepstatin A per ml). Cells were mixed with an equal volume of acid-washed glass beads and vortexed vigorously for 5 min at 4°C. Cell lysates were centrifuged at 15,900 × *g* for 10 min at 4°C, and the pellet was extracted with 2 volumes of buffer I containing 1% Triton X-100. The pellet was re-extracted once with buffer I containing Triton X-100, followed by three extractions with buffer I. After centrifugation, the pellet was extracted two times with 0.25 ml of buffer I containing 6 M urea. The supernatants from the urea-extracted pellets were pooled and diluted 1:10 with buffer I and dialyzed against 50 mM Tris hydrochloride [pH 8.0]–1 mM EDTA. Dialysates were stored in 20 mM sodium azide at 4°C. Identical procedures were used to extract ORF5 polypeptides from 0.44 ml of packed yeast cells expressing ORF5 or a control plasmid lacking HDV sequences.

Immunoblots and HDV sera. Cells from bacterial cultures induced with IPTG were pelleted and lysed, and aliquots were electrophoresed on 12% Laemmli gels (16). Immunoblots were prepared either by the method of Towbin et al. (29) (see Fig. 2) or by the method of Dunn (7) (see Fig. 3). Filters were blocked and washed either according to standard procedures described in reference 1 (see Fig. 2) or by being blocked in 5% Carnation nonfat milk (12) diluted in 1× phosphate-buffered saline (0.14 M NaCl, 2.5 mM KCl, 1.5 mM KH_2PO_4, 8 mM $Na_2HPO_4 \cdot 12H_2O$ [pH 7.4]) (see Fig. 3) before the incubation of these filters with HDV antisera and subsequent washes, as described in reference 1.

Eight HDAg-positive serum samples from patients with chronic HDV infections and four HDAg-positive serum samples from patients with acute HDV infections were generously provided by A. G. Redeker. An additional four HDAg-positive serum samples from patients with chronic infections were a gift from J. L. Gerin.

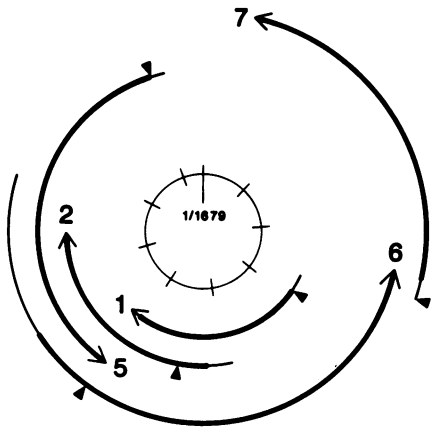
Preparation of virus and liver extracts. HDV pelleted from human sera and liver extracts from HDAg-positive liver are described in reference 1.

Immunoperoxidase staining of sectioned hepatocytes. Indirect immunoperoxide staining of HDAg-positive liver sections was done according to the method of Govindarajan et al. (9).

RESULTS

Immunological reactivity of recombinant polypeptides with HDV sera. To identify regions of the HDV genome which encode polypeptides exhibiting reactivity with HDV patient antisera, five HDV ORFs greater than 300 nucleotides from the first ATG in either the genomic or antigenomic sequence were expressed in bacteria (Fig. 1). ORF1, ORF2, ORF6, and ORF7 were expressed as fusion polypeptides with hSOD as previously reported for ORF5 (31). In the present study, ORF5 was expressed in bacteria as an unfused polypeptide by using the phSOD vector (see Materials and Methods) which was modified by the insertion of a synthetic linker designed to terminate translation after the hSOD-coding sequence and to reinitiate translation at the first ATG of the adjacent HDV sequence. Lysates of bacterial cultures expressing each of the ORFs were electrophoresed on 12% Laemmli gels (16), immunoblotted onto nitrocellulose (29), and incubated with individual antisera from 12 different patients with chronic HDV infections. Only the ORF5-encoded polypeptides bound to HDV antisera from chronically infected individuals in this type of analysis (see one representative immunoblot shown in Fig. 2). Similar immunoblots incubated with individual antisera from four different patients with acute HDV infections yielded identical results (data not shown). Although ORF5-encoded polypeptides bound to antibodies in each of the 12 antisera from HDV-infected individuals, these polypeptides did not bind to antibodies in antisera from uninfected individuals (data not shown; see reference 31). Long exposures of the autoradiogram shown in Fig. 2 failed to reveal weakly immunoreactive polypeptides encoded by any of the ORFs. The presence of the predicted ORF1, ORF2, ORF6, and ORF7 hSOD fusion polypeptides was demonstrated by positive reactivity with rabbit anti-hSOD polyclonal antibodies (data not shown). Although the ORF1-hSOD fusion polypeptide was expressed poorly in bacteria, it could still be detected with the rabbit anti-hSOD serum.

Comparison of the recombinant ORF5 polypeptides with viral polypeptides. The findings that hepatitis delta-specific antibodies bound to ORF5-derived polypeptides expressed in bacteria, which are approximately 27 kilodaltons (kDa) and 24 kDa, suggested that ORF5 might encode both putative viral polypeptides $p27^\delta$ and $p24^\delta$. To directly compare the recombinant ORF5 polypeptides with the putative viral polypeptides, lysates of bacteria expressing ORF5 were electrophoresed in lanes adjacent to extracts of pelleted HDV or extracts of HDAg-positive liver on 12% Laemmli sodium dodecyl sulfate gels, immunoblotted onto nitrocellulose (7, 16), and incubated with antiserum from a patient



orf (reading frame)		COORDINATES OF orf	COORDINATES OF EXPRESSED REGION
1	(2)	539 - 1033	578 - 1014
2	(1)*	786 - 1253	834 - 1253
5	(1)	1619 - 1014	1598 - 1014
6	(3)*	1341 - 478	1110 - 485
7	(1)*	506 - 63	481 - 63

FIG. 1. Diagrammatic representation of HDV ORFs. All HDV ORFs greater than 300 nucleotides beginning with an ATG are aligned with the circular coordinates of the HDV genome (31). The thick lines represent the portion of each ORF expressed in bacteria (see Materials and Methods). ▲, First in-frame ATG of each ORF. →, Translation of the genomic or antigenomic strand, clockwise or counterclockwise, respectively. Coordinates of each entire ORF, the region expressed in bacteria, and the relative translational frame are compiled in table form. Note that the reading frames marked with an asterisk have changed relative to our previously published sequence (31) because of the detection of an additional G residue between positions 1113 and 1114 in the HDV RNA sequence (see Fig. 4 and 5 in reference 31). The HDV genome, therefore, consists of 1,679 nucleotides.

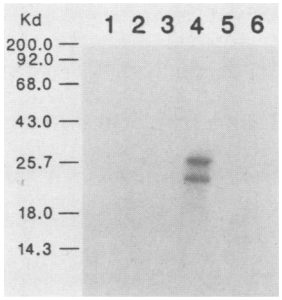


FIG. 2. Immunoblot analysis of lysates of bacteria expressing HDV ORFs. Bacterial cultures harboring control plasmid or hSOD-ORF1, -2, -6, -7, and ORF5 expression plasmids were induced with IPTG, lysed, and electrophoresed on 12% Laemmli gels (16). Lysates are in the following order: vector pSOD16cf2 (lane 1), pSOD-ORF1 (lane 2), pSOD-ORF2 (lane 3), pORF5 (lane 4), pSOD-ORF6 (lane 5), and pSOD-ORF7 (lane 6). Immunoblots were incubated with a 1:200 dilution of antiserum from a patient chronically infected with HDV followed by incubation with an ^{125}I-labeled sheep anti-human immunoglobulin G antibody (IM 133; Amersham Corp.). Molecular mass markers were obtained from Bethesda Research Laboratories (BRL SA 6040).

with a chronic HDV infection (1). Two major immunoreactive polypeptides in bacterial lysates (Fig. 3A, lane 3) appeared to comigrate with the p27$^{\delta}$ and p24$^{\delta}$ polypeptides extracted from pelleted virus or HDAg-positive liver (Fig. 3A, lanes 1 and 2, respectively). Several low-molecular-weight immunoreactive polypeptides observed in Fig. 3A, lane 3, are probably proteolytic products of p27.

The immunoreactive epitopes of both viral polypeptides p24$^{\delta}$ and p27$^{\delta}$ are encoded by ORF5. The relationship between the ORF5 polypeptides and the putative viral 24-kDa and 27-kDa polypeptides was further examined in immunological competition experiments. For these experiments, we expressed ORF5 in yeast cells as well as in bacteria. Yeast produced significantly greater amounts of the ORF5 polypeptides than did bacteria and facilitated the partial purification of the ORF5 polypeptides. Immunoblots of extracts of virus from an infectious antiserum and HDAg-positive liver were incubated with a human HDV antiserum which had been preabsorbed with either partially purified ORF5 polypeptides extracted from cultures expressing ORF5 or with control extracts from similar cultures harboring the vector plasmid lacking HDV insert DNA. The results shown in Fig. 3B and 3C indicate that the partially purified ORF5 polypeptides derived from yeast cultures but not mock extracts from control yeast cultures completely eliminated the binding of HDV-specific antibodies to the p27$^{\delta}$ and p24$^{\delta}$ polypeptides in extracts of pelleted HDV (Fig. 3B, compare lanes 1 and 2) and in HDAg-positive liver extracts (Fig. 3C, compare lanes 1 and 2). The weak, diffuse band in Fig. 3B, lane 2, appeared to represent nonspecific binding since it bound to control antisera lacking HDV marker antibodies (data not shown). The binding of HDV-specific antibodies to p27$^{\delta}$ and p24$^{\delta}$ in HDAg-positive liver extracts was not inhibited by the dialyzed urea extraction buffer (Fig. 3C, lane 5). Partially purified ORF5 polypeptides from bacterial cultures also appeared to eliminate the binding of HDV-specific antibodies to the p27$^{\delta}$ and reduced the binding of these antibodies to p24$^{\delta}$ in HDAg-positive liver extracts by at least 10-fold (Fig. 3C, compare lanes 3 and 4), on the basis of densitometry tracings of the original autoradiograms. The residual binding of the HDV antiserum to the p24$^{\delta}$ in liver is most likely due to the limiting amount of the ORF5 polypeptide in bacterial extracts.

Evidence that viral polypeptides p24$^{\delta}$ and p27$^{\delta}$ constitute the HDAg. Although p27$^{\delta}$ and p24$^{\delta}$ are the only reported polypeptides in HDV preparations which cross-react with antisera from individuals with HDV infections, there is no direct evidence that p27$^{\delta}$ and p24$^{\delta}$ are components of the nuclear delta antigen as described by Rizzetto and coworkers (23). To show that ORF5 encodes the immunoreactive epitope(s) of HDAg, we incubated sections of HDAg-positive liver with the identical, preabsorbed antisera used in Fig. 3B and assayed for antibodies binding to the nuclear HDAg by an indirect immunoperoxidase labeling method (Fig. 4). In contrast to the clear nuclear binding of antibodies against the HDAg observed in serum preabsorbed with control yeast extracts (panel A), there was no detectable binding when delta antiserum preabsorbed with the partially purified recombinant yeast ORF5 polypeptides was used (see panel B). The diluted, preabsorbed antisera used in this experiment had undetectable levels of anti-hepatitis B core antigen (HBcAg) antibodies (data not shown) and therefore did not influence the interpretation of our results. Taken

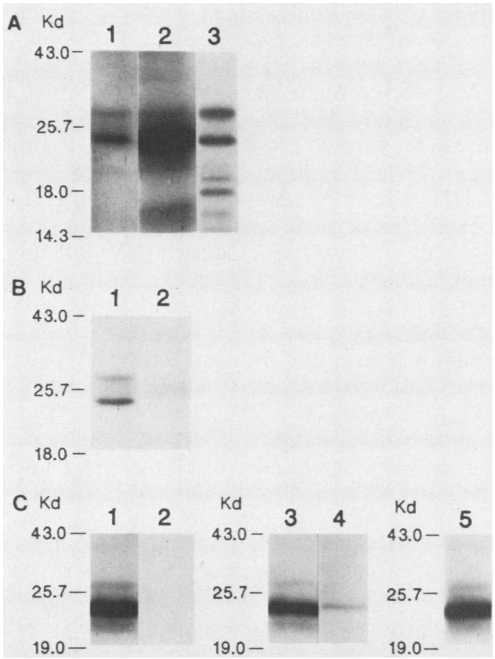


FIG. 3. Immunological analysis of the recombinant ORF5 polypeptides and the viral p24$^{\delta}$ and p27$^{\delta}$ polypeptides. (A) Immunoblot of extracts of HDV virus pelleted from an infectious serum (lane 1), HDAg-positive liver (lane 2), and a lysate from a bacterial culture expressing ORF5 (lane 3) incubated with a 1:400 dilution of a HDV antiserum (1). (B) Immunoblots of extracts of pelleted HDV incubated with a 1:1,000 dilution of an HDV antiserum preabsorbed with extracts of yeast cultures expressing either control (lane 1) or ORF5 (lane 2) expression plasmids. (C) Immunoblots of HDAg-positive liver extracts incubated with the following antisera: lanes 1 and 2, a 1:1,000 dilution of an HDV antiserum preabsorbed with extracts of yeast cultures expressing either control (lane 1) or ORF5 (lane 2) expression plasmids; lanes 3 and 4, a 1:1,000 dilution of an HDV antiserum preabsorbed with extracts from bacterial cultures expressing either control (lane 3) or ORF5 expression plasmids (lane 4). A 1:1,000 dilution of an HDV antiserum preincubated with an equal volume of dialyzed urea extraction buffer reacted with an immunoblot of HDAg-positive liver extract. All of the immunoblots in Fig. 3 were blocked with 5% Carnation nonfat milk (12) and treated as described in Materials and Methods.

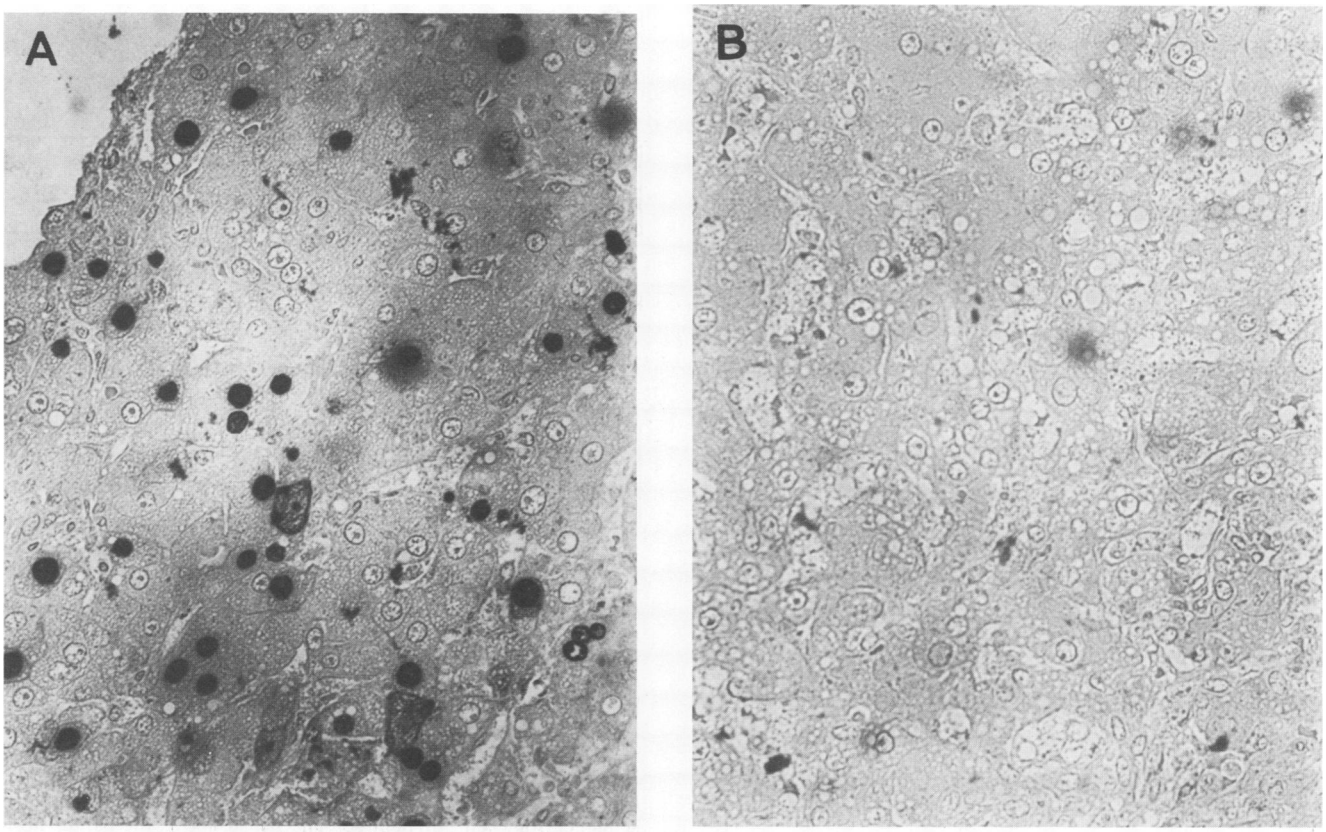


FIG. 4. Indirect immunoperoxidase staining of HDAg-positive liver sections incubated with preabsorbed delta antisera from Fig. 3. Sections of HDAg-positive liver were incubated with either a delta antiserum preabsorbed with control yeast extracts (panel A), as described for Fig. 3B and C (lanes 1), or incubated with a delta antiserum preabsorbed with partially purified yeast recombinant ORF5 polypeptide(s) (panel B), as described for Fig. 3B and C (lanes 2), followed by incubation with a peroxidase-labeled anti-human immunoglobulin G second antibody. Magnification, ×135.

together, these data indicate that the ORF5 polypeptides bind antibodies specific for both virus-associated polypeptides $p24^{\delta}$ and $p27^{\delta}$ and that these polypeptides correspond to the previously described nuclear HDAg.

DISCUSSION

The genomic and antigenomic strands of HDV could potentially encode five large polypeptides containing an amino-terminal methionine (Fig. 1). The data presented in this report indicate that only the antigenomic, ORF5-encoded polypeptides react on immunoblots with antisera from patients infected with HDV, thus suggesting that HDV may be a negative-stranded virus. However, further work is needed to establish whether ORF1, ORF2, ORF6, and ORF7 encode biologically significant polypeptides, since these ORFs may encode polypeptides which are either weakly immunogenic or nonimmunogenic or may not react on immunoblots with patient antisera, as is known to occur, for example, with some picornavirus antigens (8, 11). The possibility that ORFs less than 300 nucleotides encode functional polypeptides will also require further investigation.

The results of the immunological competition experiments shown in Fig. 3 and 4 clearly demonstrate that ORF5 encodes the immunogenic epitope(s) of both delta antigen polypeptides $p27^{\delta}$ and $p24^{\delta}$ which can be detected on immunoblots and in immunostaining assays of HDAg-positive liver sections. These data, together with the observed comigration of the viral and recombinant polypeptides on denaturing gels, strongly suggest that both $p27^{\delta}$ and $p24^{\delta}$ are entirely encoded by ORF5, although the possibility that other unidentified ORFs contribute nonimmunogenic regions of the delta antigen polypeptides cannot be excluded. It is clear, however, that the recombinant ORF5 polypeptides can be used for immunodiagnostics of antibodies to HDV in infected patients.

The HDV genomic RNA exhibits considerable sequence heterogeneity (31; unpublished observation), as is common for many viral RNAs (10). Nine sites of sequence heterogeneity have been identified in ORF5 (Fig. 5) on the basis of the sequence of four independently isolated cDNA clones from a HDV cDNA library derived from RNA extracted from a single serum (31). Because of a heterogeneity at nucleotide position 1012, the DNA sequence of cDNA clone δ115 encodes an amber (TAG) stop codon 195 amino acids from the first in-frame methionine of the predicted ORF5 polypeptide. Two other cDNA clones, both of which do not contain an amber stop codon in ORF5 but do contain a downstream TGA stop codon at nucleotide positions 954 to 956, encode a predicted ORF5 polypeptide of 214 amino acids from the putative *N*-terminal methionine. The DNA sequence of δ115 also reveals a TGA stop codon at nucleotide positions 954 to 956 of the antigenomic strand (Fig. 5). Since we have expressed the δ115 sequence in an *E. coli* amber suppressor strain, it is probable that the 27-kDa polypeptide observed in the bacterial extracts shown in Fig. 2 and 3 occurs as a result

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GlyLeuProProLeuAlaGluMetSerArgSerGluSerArgLysAsnArgGlyGlyArg
GGACTGCCGCCTCTAGCCGAGATGAGCCGGTCCGAGTCGAGGAAGAACCGCGGAGGGAGA
                  ---                                       G

GluGluIleLeuGluGlnTrpValAlaGlyArgLysLysLeuGluGluLeuGluArgAsp
GAAGAGATCCTCGAGCAGTGGGTGGCCGGAAGAAAGAAGTTAGAGGAACTCGAGAGAGAC

LeuArgLysThrLysLysLysLeuLysLysIleGluAspGluAsnProTrpLeuGlyAsn
CTCCGGAAGACAAAGAAGAAACTCAAAAAGATTGAGGACGAAAATCCCTGGCTGGGGAAC
                        G

IleLysGlyIleLeuGlyLysLysAspLysAspGlyGluGlyAlaProProAlaLysArg
ATCAAAGGAATTCTCGGAAAGAAGGATAAGGATGGAGAGGGGGCTCCCCCGGCGAAGAGG
                        C

AlaArgThrAspGlnMetGluValAspSerGlyProArgLysArgProLeuArgGlyGly
GCCCGAACGGACCAGATGGAGGTAGACTCCGGACCTAGGAAGAGGCCTCTCAGGGGAGGA

      Arg
PheThrAspLysGluArgGlnAspHisArgArgArgLysAlaLeuGluAsnLysLysLys
TTCACCGACAAGGAGAGGCAGGATCACCGACGAAGGAAGGCCCTCGAGAACAAGAAGAAG
       G

GlnLeuSerAlaGlyGlyLysAsnLeuSerLysGluGluGluGluGluLeuArgArgLeu
CAGCTATCGGCGGGAGGCAAGAACCTCAGCAAGGAGGAAGAAGAGGAACTCAGGAGGTTG

                                                          Asn
ThrGluGluAspGluArgArgGluArgArgValAlaGlyProProValGlyGlyValIle
ACCGAGGAAGACGAGAGAAGGGAAAGAAGAGTAGCCGGCCCGCCGGTTGGGGGTGTGATC
                                                            A

                                                        Leu
ProLeuGluGlyGlySerArgGlyAlaProGlyGlyGlyPheValProSerLeuGlnGly
CCCCTCGAAGGTGGATCGAGGGGAGCGCCCGGGGGCGGCTTCGTCCCCAGTCTGCAGGGA
                                                            T

                                                      Gln
ValProGluSerProPheSerArgThrGlyGluGlyLeuAspIleArgGlyAsnArgGly
GTCCCGGAGTCCCCCTTCTCTCGGACCGGGGAGGGGCTGGACATCAGGGGAAACCGGGGA
                                                            A

     END
PheProTrpAspIleLeuPheProAlaAspProProPheSerProGlnSerCysArgPro
TTTCCATGGGATATACTCTTCCCAGCCGATCCGCCCTTTTCTCCCCAGAGTTGTCGACCC
      A                         A
      *

GlnEND
CAGTGA
```

FIG. 5. Nucleotide sequence and predicted amino acid sequence of HDAg. The nucleotide sequence of ORF5 and the predicted amino acid sequence of the ORF5 polypeptide is shown above. Single nucleotide changes in the sequence of different HDV cDNA clones are given underneath the ORF5 nucleotide sequence, whereas amino acid substitutions resulting from these nucleotide changes are given above the predicted ORF5 amino acid sequence. Note that the sequence data in this figure reflects the modified HDV genome sequence described for Fig. 1. *, The heterogeneity found at nucleotide position 1012 (31) of δ115 which results in the presence of an amber stop codon in this clone. The dashed line indicates the location of the putative initiator methionine.

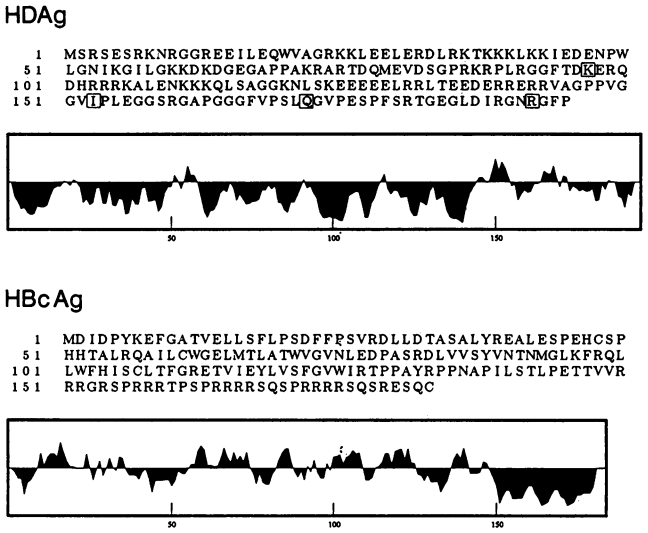

FIG. 6. Primary amino acid sequence and hydrophobicity profiles of the HDAg and the HBcAg. The predicted primary amino acid sequence of the ORF5 polypeptide beginning with the first methionine and ending with the first in-frame termination codon and the HBcAg (30) are shown in the upper and lower panels, respectively. Other sequenced delta cDNA clones from the same initial cDNA library (31) which overlap with ORF5 predict the following amino acid substitutions for the boxed amino acids: K→R, position 97; I→N, position 153; Q→L, position 172; and R→Z, position 192. Hydrophobicity profiles for the predicted ORF5 amino acid sequence and the HBcAg are shown beneath the primary amino acid sequence for each polypeptide. The peaks above the center horizontal line represent the magnitude of hydrophobicity as predicted by the rules of Kyte and Doolittle (15).

of partial host cell suppression of the first stop codon after amino acid position 195, whereas the 24-kDa polypeptide most likely represents the translation product of the ORF5 mRNA sequence terminating at that TAG. Although, the exact mechanism(s) by which $p24^{\delta}$ and $p27^{\delta}$ are generated in vivo is not known, the following three models could explain the origin of $p27^{\delta}$ and $p24^{\delta}$. (i) $p27^{\delta}$ undergoes proteolytic processing to generate $p24^{\delta}$. (ii) A heterogeneous population of RNA genomes exists such that $p24^{\delta}$ and $p27^{\delta}$ are the translation products of two different mRNAs transcribed from distinct genomes. (iii) A translational suppression of the pδ115 TAG stop codon may occur in vivo such that both $p27^{\delta}$ and $p24^{\delta}$ are synthesized from a single template. Experiments designed to distinguish between these possibilities are presently in progress. Interestingly, a poly(A) addition signal is found adjacent to the downstream ORF5 TGA stop codon (31). The possibility that the HDAg mRNA is polyadenylated is consistent with the findings of Chen et al. (4), who observed an 0.8-kb antigenomic RNA species in the liver of infected chimpanzees.

Since both the HDAg and HBcAg are contained within particles coated with hepatitis B surface antigen, we investigated the structural relationship between the ORF5-encoded HDAg and HBcAg (30) by comparing the predicted primary amino acid sequences and hydrophobicity characteristics of both polypeptides (Fig. 6). There is no apparent amino acid sequence homology between the HDAg and HBcAg, and although both proteins show a high degree of basicity, probably reflecting their interaction with viral nucleic acid, the distribution of basic amino acid residues is strikingly different for each protein. Whereas HBcAg has a very basic carboxy terminus, the predicted HDAg amino acid sequence is weakly basic in the carboxy terminus but ~30% of the first 143 amino acids consists of evenly distributed basic residues. The hydrophobicity profiles of the predicted amino acid sequence of HDAg and HBcAg are also very distinct (Fig. 6). Along with the findings that the HBV 42-nm Dane particles have a different relative composition of the hepatitis B surface antigen polypeptides than those found in the 36-nm HDV particles (2), our data support the idea that the HDAg and HBcAg are packaged in viral particles which have distinctly different architectures.

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