JOURNAL OF VIROLOGY, June 1986, p. 945–950
0022-538X/86/060945-06$02.00/0

Vol. 58, No. 3

Hepatitis Delta Virus: Protein Composition of Delta Antigen and Its Hepatitis B Virus-Derived Envelope

FERRUCCIO BONINO,[1] KLAUS H. HEERMANN,[2] MARIO RIZZETTO,[1] AND WOLFRAM H. GERLICH[2]*

Department of Gastroenterology, S. Giovanni Hospital "Molinette," Turin, Italy,[1] and Department of Medical Microbiology, University of Göttingen, D-3400 Göttingen, Federal Republic of Germany[2]

Received 23 September 1985/Accepted 18 February 1986

Hepatitis delta virus (HDV)-associated particles were purified from the serum of an experimentally infected chimpanzee by size chromatography and by density centrifugation. Hepatitis delta antigen (HDAg) was detected after mild detergent treatment at a column elution volume corresponding to 36-nm particles and banded at a density of 1.25 g/ml. The serum had an estimated titer of 10^9 to 10^{10} HDV-associated particles and had only a 10-fold excess of hepatitis B surface antigen (HBsAg) not associated with HDAg. Therefore, HDV appears to be much more efficiently packed and secreted than is its helper virus, hepatitis B virus (HBV), which is usually accompanied by a 1,000-fold excess of HBsAg. The protein compositions of the HDAg-containing particles were analyzed by immunoblotting with HDAg-, HBsAg-, and hepatitis B core antigen-specific antisera and monoclonal antibodies to HBV surface gene products. The HBsAg envelope of HDAg contained approximately 95% P24/GP27^s, 5% GP33/36^s, and 1% P39/GP42^s proteins. This protein composition was more similar to that of the 22-nm particles of HBsAg than to that of complete HBV. The significant amount of GP33/36^s suggests that the HBsAg component of the HDV-associated particle carries the albumin receptor. Two proteins of 27 and 29 kilodaltons which specifically bound antibody to HDAg but not HBV-specific antibodies were detected in the interior of the 36-nm particle. Since these proteins were structural components of HDAg and were most likely coded for by HDV, they were designated P27^d and P29^d.

Hepatitis delta virus (HDV) has been characterized as a 36-nm spherical particle containing an RNA of 1,750 bases and a specific antigen (delta antigen [HDAg]) (1, 2, 11, 15). HDV is a defective virus requiring helper functions of hepatitis B virus (HBV) for its replication (15). HBV-infected cells overproduce the viral envelope protein (hepatitis B surface antigen [HBsAg]), and this surplus material is utilized by HDV, which apparently has no envelope protein (2, 7) of its own. HDAg from serum is precipitated by an antibody to HBsAg (anti-HBs), and it is only detected after removal of the HBsAg envelope with detergents (1, 2, 11).

HBsAg is composed of three protein pairs which are coterminal. The part common to all three proteins is coded for by the *s* gene of HBV DNA, a section of 55 amino acids upstream is coded for by the pre-s_2 region, and an additional section of 108 or 119 amino acids is coded for by the pre-s_1 region. Each of the three possible proteins is facultatively glycosylated in its *s* gene part, so six HBV surface (HBs) proteins are found by gel electrophoresis (6). P24^s and its glycosylated derivative GP27^s are the major HBs proteins. GP33^s and GP36^s contain, in addition, the pre-s_2 sequence, which is always glycosylated in these proteins (14). HBV and HBsAg particles from highly viremic carriers contain more GP33/36^s than do HBsAg particles from carriers with low titers of HBV in the blood (13). Complete HBV particles also contain much of the two larger HBs proteins, P39^s and GP42^s. In contrast, the 22-nm HBsAg particles contain only traces of this protein pair (6).

One unanswered question at present is whether the envelope of HDV-associated particles resembles that of complete HBV or is more similar to that of the defective 22-nm forms of HBsAg. Another unanswered question involves the nature of the proteins forming HDAg in the interior of the particle. In the present study HDV-associated particles were purified by a novel procedure from the blood of an experimentally infected chimpanzee. The protein composition of the purified particles was studied by the immunoblot procedure with HBsAg- and HDAg-specific antibodies.

MATERIALS AND METHODS

Source of HDAg. A 7-year-old chimpanzee that was a chronic carrier of HBsAg (adw) was inoculated with serum obtained from another animal that was a chronic carrier of HBsAg (subtype ayw) during the acute phase of HDV infection. The inoculum was kindly provided by J. L. Gerin and R. H. Purcell. This chimpanzee was part of a transmission study of HDV performed by H. Schellekens and A. Ponzetto at the Primate Center, Institutes of the Division for Health Research TNO, Rijswijk, The Netherlands. Serum HDAg appeared in parallel with the peak expression of HDAg in the liver 3 weeks after inoculation and had characteristics identical to those previously reported (2, 10, 11). HDAg was detected only after treatment with detergent (Nonidet P-40; Fluka).

Antigen assays. HBsAg was assayed quantitatively by an enzyme immunoassay in microtiter plates (Behringwerke) with reference sera supplied by the Paul Ehrlich Institut, Frankfurt, Federal Republic of Germany, as the standards (5). HDAg and antibody to HDAg (anti-HD) were tested by a microtiter solid-phase radioimmunoassay (RIA) as previously reported (12). Test samples were diluted, if necessary, in 0.13 M NaCl–0.01 M sodium phosphate (pH 7.4)–10% fetal calf serum for HBsAg or in the same buffer containing 0.3% Nonidet P-40 instead of fetal calf serum for HDAg.

Purification of HDAg particles. Acute-phase serum (1.5 ml) from the HDV-infected chimpanzee was spun for 1 min at 5,000 × *g*, and the supernatant was applied to a column (1.6 by 100 cm) containing 180 ml of 6% agarose beads (Bio-Gel A5M, 200/400 mesh; Bio-Rad Laboratories). Before the first run, nonspecific absorption of proteins was blocked by

* Corresponding author.

passing 1 ml of normal human serum through the column. Elution was done at 3 ml/h and 4°C with 0.13 M NaCl–10 mM Tris hydrochloride–1 mM disodium EDTA (TNE). Fractions of 1 ml were collected and tested for HDAg and HBsAg by an enzyme immunoassay or RIA and by HBs- or HD-protein-specific immunoblots.

Peak fractions of HDAg were combined, and to 3 ml of this pool solid CsCl was added to produce a final density of 1.24 g/ml. This sample was layered on 0.7 ml of a CsCl-TNE solution (1.30 g/ml) in a centrifuge tube (13 by 51 mm). The tube was then filled completely by an overlay of TNE and centrifuged in a swinging-bucket rotor for 36 h at 38,000 rpm (160,000 × *g*) and 4°C. Fractions of 200 μl were collected from the bottom and tested for HBsAg and HDAg. Density was measured by refractometry. Peak fractions of HDAg were combined and stored for not longer than 1 week at 4°C. A sample of HBsAg without HDAg was centrifuged in the same way, but the density of the sample was adjusted to 1.20 g/ml.

Immunoblotting. Samples of 20 μl of chromatography eluate or 10 μl of density centrifugate were analyzed as described previously (6). For electrophoresis of the reduced, denatured protein, a 13% polyacrylamide gel was used (13). For the detection of HDAg-reactive protein, the membrane with the electropherogram was incubated with the serum of a patient with a chronic HDV infection; the serum was diluted 1:500 in 20% fetal calf serum. This serum had an anti-HD titer of 10^6 in the RIA, whereas antibodies to hepatitis B core and e antigens (anti-HBc and anti-HBe, respectively) had titers of 10^3 and 10^2, respectively. The serum contained HBsAg(ayw) but no HDAg. Anti-HD immunoglobulin G (IgG)-binding protein bands were stained enzymatically with peroxidase-labeled antibody against human IgG and diaminobenzidine as described previously (6). As a negative control antibody, an anti-HD-negative serum (F 502) from a healthy HBsAg carrier was used. This serum had an anti-HBc titer of 1:40,000 and an anti-HBe titer of 1:100,000. Purified hepatitis B core antigen (HBcAg) from an *Escherichia coli* strain which was transformed by gene *c* of HBV (W. Gerlich, unpublished data) served as the control antigen.

For the detection of HBs proteins, the stained membranes were incubated for 1 h at 37°C in commercially available ^{125}I-labeled anti-HBs reagent used for the assay of HBsAg (Abbott Laboratories or Electro-Nucleonics, Inc.). The washed membranes were autoradiographed overnight as described previously (13).

RESULTS

Purification of HDAg particles. Serum containing HDAg was obtained from an acutely infected chimpanzee. Owing to the time of blood sampling, it appeared likely that some anti-HD, mainly of the IgM class, was already present in the sample. The first purification step was to remove the majority of serum proteins and any anti-HD. Beads of 6% agarose were used for the separation, as this material differentiates well particles between 5 and 45 nm (3). The elution profiles of HDAg and HBsAg are shown in Fig. 1. No peak of HBsAg was detected at the voided volume of the column. This result suggested that HBsAg particles larger than 40 nm, i.e., HBV and HBsAg filaments, were not present in detectable amounts (4). There were, however, three HBsAg-reactive particle sizes discernible at 33, 35, and 41% of the total volume of the column (Fig. 1). These elution volumes corresponded approximately to spherical particles 36, 32, and 22 nm in diameter (3). The 36-nm particles formed only a shoulder in front of the more prominent elution peak of 32-nm particles.

HDAg activity was associated with an apparently homogeneous population of 36-nm particles and was probably contained within the 36-nm particles of the HBsAg activity. On the basis of this assumption, the number of HDV-associated particles and their relative proportions in HBsAg could be estimated. The sample contained approximately 50 ng of HBsAg bound to HDAg particles per ml, 200 ng of HBsAg bound to 32-nm particles, and 500 ng of HBsAg bound to 22-nm particles. Assuming a molecular weight of 10^7 for HDV-associated particles, a titer of 10^9 to 10^{10}/ml was observed in the examined sample.

The peak fraction of HDAg activity (fractions 59 to 61, pool I, Fig. 1), and, for comparison, HBsAg particles almost devoid of HDAg activity (fractions 64 to 66, pool II, Fig. 1) were centrifuged in CsCl gradients to density equilibrium (Fig. 2). The HDAg-containing particles formed an apparently homogeneous zone at densities between 1.24 and 1.26 g/cm^3, with a maximum at 1.250 g/cm^3. The HDAg activity of the fractions in the RIA was three times higher than that in

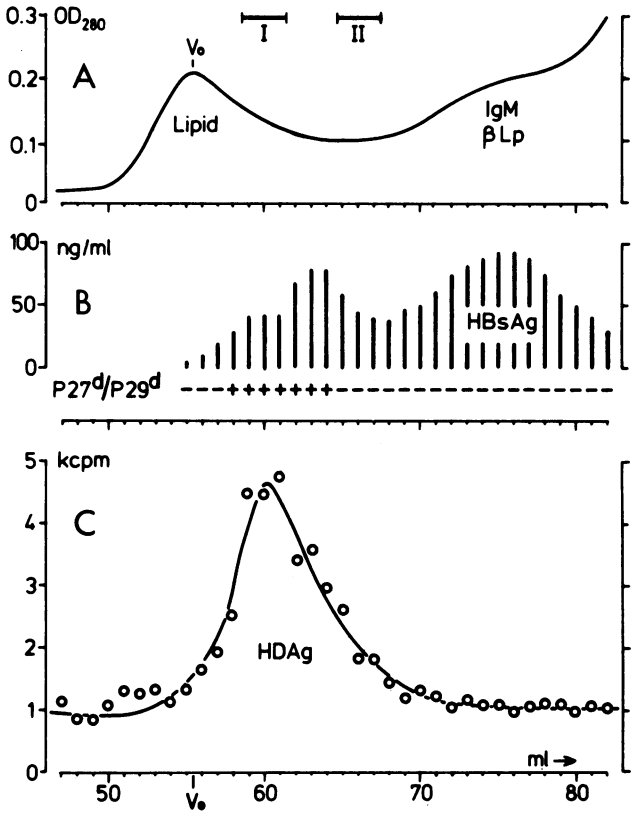

FIG. 1. Size chromatography of HDV-associated particles. HDAg-positive serum was passed through a column of 6% agarose beads as described previously (4). The optical density at 280 nm (OD_{280}) of the eluate was continuously recorded (A), revealing the position of major serum components (3) like lipid vesicles, β-lipoprotein (βLp), or IgM. The elution profile of HBsAg (B) was assayed in 1:10 dilutions of the fractions, and that of HDAg (C) was assayed after the addition of 0.3% Nonidet P-40. The result of the anti-HD-specific immunoblot (see also Fig. 3A) is recorded in panel B. V_0 marks the voided volume of the column. HDAg-positive peak fractions were combined to pool I; slightly smaller HBsAg particles without HDAg were combined to pool II.

the size chromatrography eluate. This was due to the concentrating effect of the isopycnic banding. No apparent antigenicity losses were observed directly after the centrifugation, but prolonged storage for more than 1 week at 4°C resulted in a significant loss of activity. HBsAg in pool I formed two density peaks; a sharp peak was observed at 1.250 g/cm^3, together with HDAg, and two-thirds of the activity was distributed heterogeneously between densities of 1.24 and 1.19 g/cm^3, with a maximum at 1.232 g/cm^3. HBsAg in pool II had a density distribution similar to that of the lighter subfraction in pool I. The sharp banding of HBsAg activity in pool I at a density of 1.250 g/cm^3 was missing in pool II (Fig. 2B). Thus, most of the HBsAg activity in fractions 10 and 11 of pool I was associated with HDAg-containing particles. The HDAg activity in the RIA (up to 15,000 cpm/30-μl assay) was consistent with the estimated titer of HDV-associated particles, as it was derived from the amount of HBsAg.

Anti-HD-binding proteins in purified HDV-associated particles. The limited amount of starting material and the low concentration of HDV proteins did not allow a complete purification of the HDAg particles or the usual biochemical characterization of proteins. However, some HDV proteins could be identified by the immunoblot procedure.

The relevant fractions from size chromatography and density centrifugation were subjected to sodium dodecyl sulfate gel electrophoresis, and the separated polypeptide chains were transferred to a membrane. The membrane was incubated with diluted human anti-HD antiserum. The binding of IgG from this serum to proteins on the membrane was subsequently detected by peroxidase-labeled anti-IgG and enzymatic staining (Fig. 1B and 3A).

Enzymatic staining revealed a protein of 53 kilodaltons (kDa) in all tested fractions. The appearance and intensity of this protein band did not correlate with HDAg or HBsAg activity. The HDAg-positive fractions from size chromatography had two bands of approximately 27 and 29 kDa (Fig. 1 and 3A). These two proteins were also visible in the HDAg-positive fractions from density centrifugation (Fig. 2A and 4A). HDAg-negative fractions from size chromatography or density centrifugation did not have these two bands.

The anti-HD serum which was used for the detection of P27^d and P29^d was from a patient chronically infected with both HDV and HBV. In addition to anti-HD, it also contained anti-HBc. To exclude the possibility that P27^d and P29^d were HBV core proteins of unusual size or nonspecific IgG-binding proteins, we controlled the specificity of enzymatic staining by the following experiment (Fig. 5). HDAg proteins from combined fractions 10 and 11 and, for comparison, HBV core proteins expressed in *E. coli* were analyzed in duplicate by the immunoblot procedure. One set of membranes (Fig. 5A and C) was incubated with the anti-HD antiserum. The other set of membranes (Fig. 5B and D) was incubated under the same conditions with an anti-HD-negative human serum from a chronic carrier of HBsAg. This serum was known to contain high titers of anti-HBc and anti-HBe. Both antisera reacted with the HBV core protein of 22 kDa and its smaller degradation product of 20 kDa, thus proving the efficiency of immunoblot staining for any detectable core protein (Fig. 5C and D). In keeping with its 100-fold-lower anti-HBc titer, the anti-HD antiserum stained P22^c much more weakly than did the control anti-HBc antiserum. No reaction was seen between P27^d or P29^d and the anti-HBc antiserum (Fig. 5B). These findings excluded the possibility that P27^d and P29^d were larger variants of the HBV core protein or that they were nonspecifically stained components. In contrast, the 53-kDa band was stained by both human antisera (Fig. 5A and B), so it is very unlikely that this band has a specific relationship to HDV.

The weak intensity of the HDV protein bands as compared with the P22^c band suggests that in the experiment in Fig. 5 the amount of HDAg was the limiting factor, since the anti-HD titer of the HD-antiserum was comparable to the anti-HBc titer of the control serum. Despite its 100-fold-lower anti-HBc titer, the anti-HD antiserum still produced a stronger staining of P22^c than it did of HDV proteins.

Envelope proteins in HDV-associated particles. After enzymatic staining, immunoblot membranes were further incubated with ^{125}I-labeled anti-HBs. The binding of ^{125}I-labeled anti-HBs was detected by autoradiography (Fig. 3B and 4B). All HBsAg-positive fractions from size chromatography contained the known P24^s and GP27^s as major HBs proteins and GP33^s and GP36^s as minor HBs proteins in a similar relative proportion. A direct comparison with purified 22-nm particles from a viremic HBV carrier (Fig. 3B, rightmost

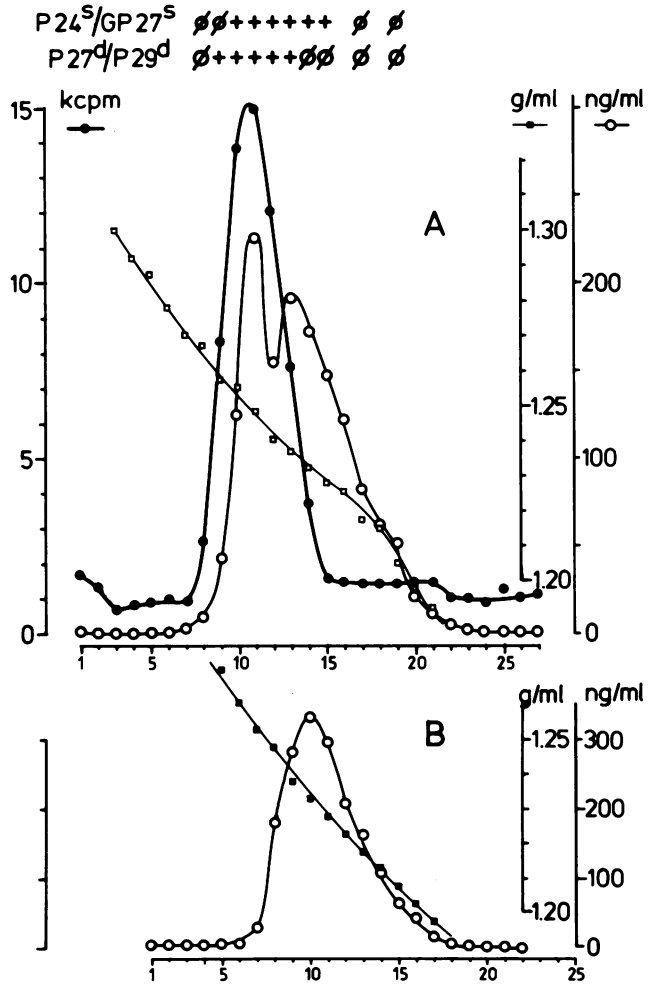

FIG. 2. Density distribution of HDAg and HBsAg. HDAg-positive (pool I, A) and -negative (pool II, B) fractions from Fig. 1 were subjected to isopycnic banding as described in the text, and HDAg (●) or HBsAg (○) was assayed in the fractions of the density gradient. Fractions 8 to 19 of pool I (A) were also studied by immunoblotting. The detection of HBs and HDV proteins is recorded at the top.

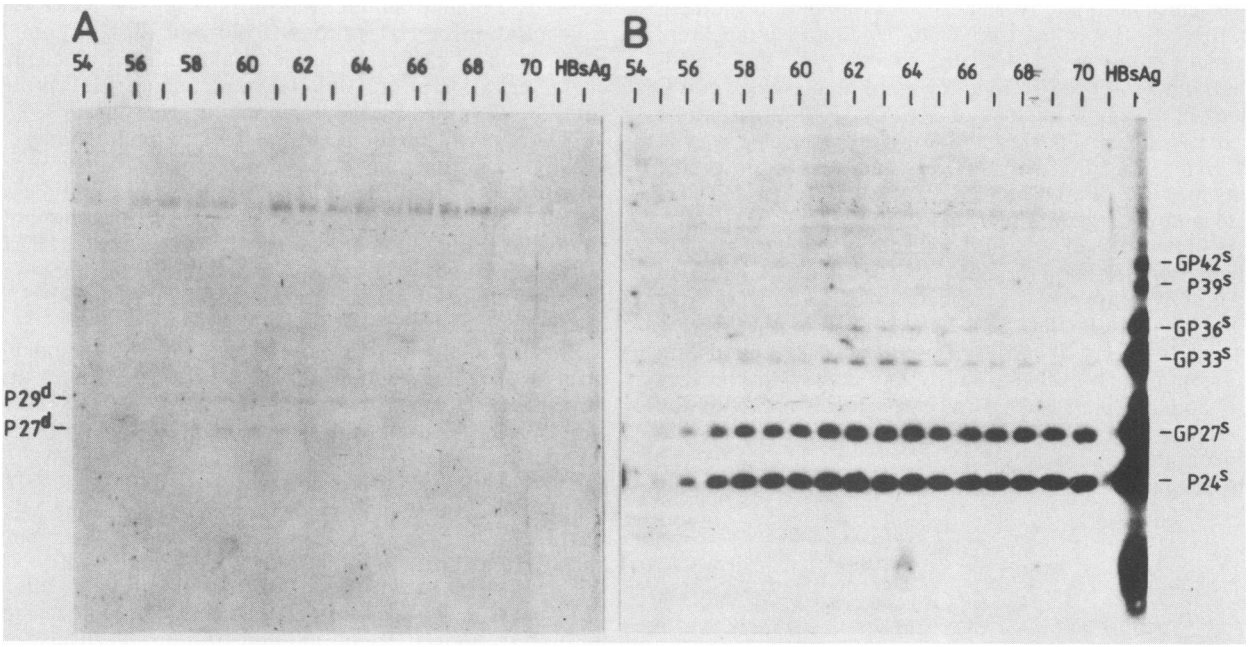

FIG. 3. Detection of HDAg (A) and HBsAg (B) proteins in the size chromatography eluate. Samples of the fractions from Fig. 1 were subjected to denaturing gel electrophoresis, the separated proteins were transferred to a membrane, and the membrane was stained first with HDAg-specific reagents as described in the text (A) and thereafter reacted with radioactive antibody specific for HBsAg. The autoradiogram showing the HBs proteins of the same membrane as that in panel A is shown in panel B.

lane) revealed that the HBsAg of the HDV-infected chimpanzee contained relatively less GP33^s and GP36^s. The proportion of GP33^s and GP36^s was nevertheless higher than in purified 22-nm particles from nonviremic HBV carriers (13). In purified particles from such carriers, GP33^s and GP36^s make up less than 1% of the total HBs proteins. P39^s and GP42^s were not detected by the radioimmunoblot in the HDAg-positive samples. HBsAg-positive samples, however, contained at least one P39^s or GP42^s molecule per particle, since HBsAg bound efficiently to a monoclonal antibody against the pre-s_1 part of P39/GP42^s (Table 1). No significant difference in the binding of HBsAg to this antibody was found between the HDV-associated particles and the HDAg-negative HBsAg particles.

Direct comparison of the enzymatically stained bands of the HDV proteins and the autoradiographic bands of the HBs proteins showed that the smaller HDAg protein, P27^d, comigrated during sodium dodecyl sulfate gel electrophoresis with GP27^s. The other HDAg protein was apparently 2 kDa larger. Thus, the two proteins may be referred to as P27^d and P29^d. It is very unlikely that P27^d and P29^d are related to the HBV envelope proteins. The anti-HD antiserum never stained proteins of HBsAg particles from the same source when the particles were devoid of HDAg. On

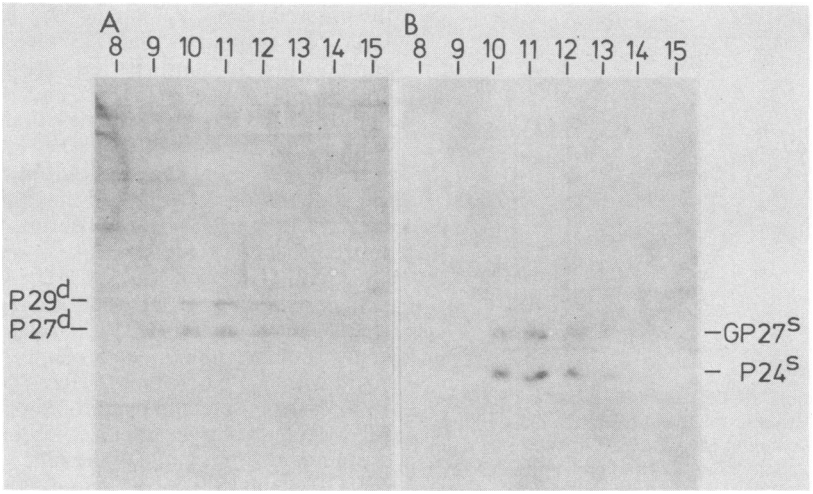

FIG. 4. Detection of HDAg (A) and HBsAg (B) proteins in the density gradient. Samples of the fractions from Fig. 2A were analyzed as described in the legend to Fig. 3.

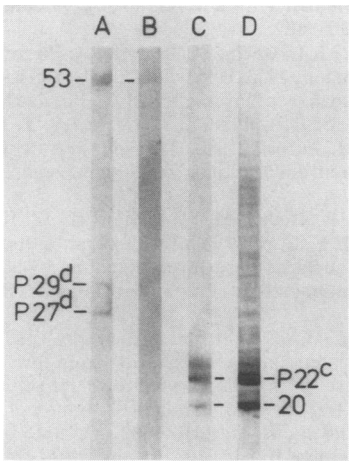

FIG. 5. Specificity of the enzyme immunoblot for HDV proteins. Purified HDV-associated particles (combined fractions 10 and 11 of Fig. 2A), or, for the control, 0.5 μg of HBcAg from *E. coli*, were denatured, and the electrophoretically separated proteins were transferred to a membrane. Membrane strips were enzymatically stained as described in the text with either human anti-HD serum or human anti-HBc serum. Lanes: A, HDAg stained with anti-HD; B, HDAg stained with anti-HBc; C, HBcAg stained with anti-HD; D, HBcAg stained with anti-HBc. Note the absence of the $P22^c$ band in lanes A and B and the absence of the $P27^d$ and $P29^d$ bands in lanes B to D.

the other hand, the anti-HBs reagent was immunoaffinity purified with HBsAg 20-nm particles. It never reacted with a 29-kDa band, and it did not detect the 27-kDa band in the most purified fraction (fraction 9) of HDAg particles, with which the anti-HD antiserum still gave a strong reaction in the immunoblot (Fig. 4).

DISCUSSION

Size chromatography appears to be an efficient procedure for purifying HDV-associated particles from the majority of serum proteins and any anti-HD. In combination with density centrifugation, it was also able to remove the HBsAg particles which did not contain HDAg. Surprisingly, these HBsAg particles contained a subfraction of unusual size (32 nm) and density (1.23 g/ml) in addition to the well-known particles of 20 nm and 1.20 g/ml. The significance of these particles is unknown. Their occurrence is probably not related to the HDV infection.

The purification of HDV-associated particles from the 10-fold excess of HDAg-negative HBsAg particles allowed an approximate quantitation of their titer via the amount of HBsAg on their envelope. The titer of 10^9 to 10^{10}/ml was found to be higher than the highest recorded HBV titer. On the other hand, the HBsAg concentration in the HDV serum specimen was lower by a factor of 10^2 than those in most samples from highly viremic HBV carriers (5). Apparently, HDV is much more efficiently packed and secreted than is HBV. HDV infectivity titers as high as 10^{11} have been reported in serum samples from other acutely infected chimpanzees (9). Although the infectivity titer in the specimen examined here is unknown, it is likely that most of its HDV-associated particles represented infective HDV virions, since the titer of these particles was in the same range as the infectivity titer of particles from comparable serum samples.

TABLE 1. Binding of HBsAg to a monoclonal antibody against the pre-s_1 sequence (6)

Sample	HBsAg (ng/ml)	Anti-pre-s_1 OD_{492}[a]
HDV-associated particles, fractions 10 and 11	35	1.19
HBsAg pool I, fraction 14	30	0.70
HBsAg pool II, fraction 10	60	1.02
Negative control		0.02

[a] The monoclonal antibody was adsorbed to microtiter plates. The binding of HBsAg was detected by the addition of peroxidase-labeled polyvalent anti-HBs (Behringwerke) and a subsequent color reaction with *o*-phenylenediamine. OD_{492}, Optical density at 492 nm.

The HBsAg envelope of HDV contains apparently all known HBs proteins. However, HDV does not require the large proportion of $P39/GP42^s$ which is always found in the envelope of complete HBV (6). It appears that HDAg is efficiently enveloped by HBs proteins, because they are usually found in HBsAg 20-nm particles. This finding is in agreement with the clinical observation that HDAg production and HDV maturation proceed in patients without the efficient production of complete HBV virions (15).

Connected with persistent massive HBV viremia is also a high amount of $GP33/36^s$ (13). A significant amount of $GP33/36^s$ was found in HDV-associated particles, and it may be also an essential component for massive HDV viremia. It is known that the pre-s_2 sequence of $GP33/36^s$ binds glutaraldehyde-treated human albumin (8). Thus, HDV-associated particles most likely carry the HBsAg-associated albumin receptor.

The two newly identified anti-HD-binding proteins, $P27^d$ and $P29^d$, are probably major structural components of HDAg resident within HDV-associated particles. The amount of HDAg analyzed was probably too small for minor components to be detected. The specific enzymatic staining of the two proteins by the serum of a patient infected with HDV and HBV and the absence of staining by an HBV patient's serum strongly suggest that the two proteins are coded for by the genome of HDV and not by the helper virus or by the host. At the present state of knowledge it remains open whether the two proteins are coded for by completely different parts of the HDV genome. In that case, most of the HDV RNA would be occupied by their coding sequences. Alternatively, the two proteins could be derived from one coding sequence by posttranscriptional or posttranslational modification.

In view of the small amounts of HDAg-positive serum available, it is unlikely that the nature of the two HDV proteins can be solved by direct structural analysis. More knowledge will be obtained when the nucleotide sequence of a cloned HDV genome becomes available and when peptide-specific antibodies identify the coding sequence(s) of the two HDAg proteins.

ACKNOWLEDGMENTS

This work was sponsored in part by a contract between the Regional Government of Piemonte, Assessorato alla Sanita, and the Department of Gastroenterology, S. Giovanni Hospital, "Molinette," Turin, Italy.

We thank R. Thomssen for support of the studies in Göttingen.

LITERATURE CITED

1. **Bonino, F., B. Hoyer, G. Ford, J. W.-K. Shih, R. H. Purcell, and J. L. Gerin.** 1981. The delta agent: HBsAg particles with antigen and RNA in the serum of an HBV carrier. Hepatology (Balti-

more) 2:127–131.
2. **Bonino, F., B. Hoyer, W.-K. Shih, M. Rizzetto, R. H. Purcell, and J. L. Gerin.** 1984. Delta hepatitis agent: structural and antigenic properties of the delta-associated particle. Infect. Immun. **43**:1000–1005.
3. **Gerlich, W.** 1971. Determination of particle size by gel chromatography with Sepharose 6B. Z. Naturforsch. Teil B Org. Chem. **26**:1040–1044.
4. **Gerlich, W., C. Gauhl, and G. May.** 1975. Separation of morphological types of hepatitis B antigen. Zentralbl. Bakteriol. Mikrobiol. Hyg. Abt. 1 Orig. A **232**:189–198.
5. **Gerlich, W., and R. Thomssen.** 1975. Standardized detection of hepatitis B surface antigen: determination of its serum concentration in weight units per volume. Dev. Biol. Stand. **30**:78–87.
6. **Heermann, K. H., U. Goldmann, W. Schwartz, T. Seyffarth, H. Baumgarten, and W. H. Gerlich.** 1984. Large surface proteins of hepatitis B virus containing the pre-*s* sequence. J. Virol. **52**:396–402.
7. **Hoyer, B., F. Bonino, A. Ponzetto, K. Denniston, J. Nelson, R. H. Purcell, and J. L. Gerin.** 1983. Properties of delta associated ribonucleic acid. Prog. Clin. Biol. Res. **143**:91–99.
8. **Machida, A., S. Kishimoto, H. Ohnuma, K. Baba, Y. Ito, H. Miyamoto, G. Funatsu, K. Oda, S. Usuda, S. Togami, T. Nakamura, Y. Miyakawa, and M. Mayumi.** 1984. A polypeptide containing 55 amino acid residues coded by the pre-s region of hepatitis B virus deoxyribonucleic acid bears the receptor for polymerized human as well as chimpanzee albumins. Gastroenterology **86**:910–918.
9. **Purcell, R. H., J. L. Gerin, M. Rizzetto, A. Ponzetto, F. Bonino, and W. T. London.** 1983. Experimental transmission of the delta agent to chimpanzees. Prog. Clin. Biol. Res. **143**:79–90.
10. **Rizzetto, M., M. G. Canese, J. L. Gerin, W. T. London, D. L. Sly, and R. H. Purcell.** 1980. Transmission of the hepatitis B virus associated delta antigen to chimpanzees. J. Infect. Dis. **141**:590–602.
11. **Rizzetto, M., B. Hoyer, M. G. Canese, J. W.-K. Shih, R. H. Purcell, and J. L. Gerin.** 1980. Delta agent: association of delta antigen with hepatitis B surface antigen and RNA in serum of δ-infected chimpanzees. Proc. Natl. Acad. Sci. USA **77**: 6124–6128.
12. **Rizzetto, M., J. W.-K. Shih, and J. L. Gerin.** 1980. The hepatitis B virus associated δ-antigen: isolation from liver, development of a solid phase radioimmunoassay for δ antigen and anti-δ and partial characterization of δ antigen. J. Immunol. **125**:318–324.
13. **Stibbe, W., and W. H. Gerlich.** 1982. Variable protein composition of hepatitis B surface antigen from different donors. Virology **123**:436–442.
14. **Stibbe, W., and W. H. Gerlich.** 1983. Structural relationships between minor and major proteins of hepatitis B surface antigen. J. Virol. **46**:626–628.
15. **Verme, G., F. Bonino, and M. Rizzetto.** 1983. Viral hepatitis and delta infection. Prog. Clin. Biol. Res. **143**:1–421.