Core Particles of Hepatitis B Virus and Ground Squirrel Hepatitis Virus

I. Relationship Between Hepatitis B Core Antigen- and Ground Squirrel Hepatitis Core Antigen-Associated Polypeptides by Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis and Tryptic Peptide Mapping

MARK A. FEITELSON,[†] PATRICIA L. MARION, AND WILLIAM S. ROBINSON*

Department of Medicine, Stanford University, Stanford, California 94305

Received 30 December 1981/Accepted 15 April 1982

The relationships among the core antigen polypeptides of hepatitis B virus (HBV) and ground squirrel hepatitis virus (GSHV) were studied using sodium dodecyl sulfate-polyacrylamide gel electrophoresis and tryptic peptide mapping. The major core antigen polypeptides of liver-derived HBV (p22) and GSHV (p20.5) shared 56% of the spots in their peptide maps. Comparison of hepatitis B core antigen (HBcAg) p19 or ground squirrel hepatitis core antigen (GSHcAg) p16.5 with their respective major polypeptides indicated that these components probably resulted from cleavage of the major polypeptide of each virus. Other polypeptides smaller than the major component of each virus were often faint on polyacrylamide gels and probably resulted from the cleavage or degradation of components larger than p22 of HBcAg or p20.5 of GSHcAg, since their peptide maps contained spots unique to these high-molecular-weight components. p26 of GSHcAg and p27.5 of HBcAg shared approximately two-thirds of the spots on their peptide maps with those of their respective major core polypeptides. Furthermore, p37.5 of GSHcAg and p40 of HBcAg shared about 60% homology with their respective major polypeptides, and also shared many of the spots that were unique to p26 of GSHcAg or p27.5 of HBcAg but were not found in the peptide map of their respective core antigen polypeptides. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis bands larger than 40,000 daltons were variably present, and peptide mapping indicated that these were aggregates of various smaller core antigen-associated polypeptides. The results suggest that p40 of HBcAg and p37.5 of GSHcAg are the largest unique polypeptides in these core particles, and that they are encoded for by the genome of each virus. That a subset of the spots unique to p40 or p37.5 was also found in p27.5 of HBcAg or p26 of GSHcAg, respectively, as compared to the major core polypeptides, also suggests that p27.5 and p26 are unique proteins encoded by the genome of each virus. It is proposed that the core antigen gene of each virus is larger than that which would encode the major polypeptide of each virus, and that the genetic organizations of the core genes of HBV and GSHV are very similar.

The presumptive virion of hepatitis B virus (HBV), or Dane particle (7), and Dane-like particles recently described in the ground squirrel hepatitis virus (GSHV) system (18) are large spherical structures 40 to 45 nm in diameter found in the blood of many individuals infected by the respective virus. The envelope of each contains surface antigen determinants which have been shown to be serologically cross-reactive and polypeptides that share at least some structural relatedness (8, 13, 18). Removal of the envelope with nonionic detergent exposes a vi-

rus-associated core antigenic system which is distinct from the surface antigen of each virus (2, 18). The core antigen has been found associated with 27-nm hepatitis B core antigen (HBcAg) and ground squirrel hepatitis core antigen (GSHcAg) particles isolated from virions or infected livers (2, 4, 14, 15, 18). Studies directed toward the characterization of the polypeptides associated with core particles have identified a major component at 19,000 to 20,000 daltons and several high-molecular-weight components which have varied in both number and size (6, 15, 21). Furthermore, e antigenic activity (the third serologically unique system in HBV) has recently been found in core particles upon treat-

[†] Permanent address: The Institute for Cancer Research, Fox Chase Cancer Center, Philadelphia, PA 19111.

ment with sodium dodecyl sulfate (SDS) or limited proteolysis (5, 18, 22, 23, 30) and localized to the major polypeptide (p19) of HBcAg (26). The core particles, in addition, possess an endogenous DNA polymerase activity which makes fully double stranded the partially single-stranded genome within the core of each virus (16, 18, 24, 25). These core particles also have an associated protein kinase activity which phosphorylates many of the polypeptides in cores (1, 9). For HBcAg and GSHcAg, the major sites of phosphorylation are similar both in the major polypeptide and in the minor polypeptides of higher and lower molecular weight, indicating that although important differences exist in the core polypeptides of HBcAg and GSHcAg (this study), the sites of phosphorylation are conserved.

Further development of the GSHV-ground squirrel system as an animal model for studying virus gene expression after infection can, in part, be accomplished by characterizing and comparing the various gene products of HBV and GSHV. Here the polypeptides of liver-derived core antigen particles are compared by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and tryptic peptide mapping.

MATERIALS AND METHODS

Isolation of core particles from liver. HBV-infected human livers were screened for the presence of core by indirect immunofluorescence, and highly positive livers were selected for core isolation. GSHV-infected ground squirrel livers were obtained from wild-caught animals subsequently shown to have high DNA polymerase levels in serum. Core particles were isolated by a modification of a method designed by Wolfram Gerlich. Several grams of human liver or several hundred milligrams of ground squirrel liver stored at -70°C were thawed and rinsed in ice-cold phosphate-buffered saline (PBS), pH 7.5 (Baker; analyzed reagents). Thirty percent homogenates were prepared in a Waring blender with ice-cold PBS. Connective tissue and most cellular debris were removed from the homogenate by centrifugation at 10,000 rpm for 20 min at 4°C in a Sorvall GSA rotor. Supernatants were made up to a density of 1.19 g/ml with CsCl (KBI), and 10-ml samples were layered upon freshly prepared discontinuous CsCl gradients (made up in PBS, pH 7.5) with density steps of 1.40, 1.35, 1.30, and 1.25 g/ml added in equal volumes. The volume of the homogenate samples usually did not exceed 25% of the discontinuous gradient volume. CsCl density equilibrium centrifugation was carried out at 24,000 rpm for 60 h at 10°C in an SW27 rotor. Fractions collected by inserting a small tube to the bottom of each gradient were tested by enzyme-linked immunosorbent assays specific for each antigen (W. Gerlich, G. H. Scullard, and W. S. Robinson, unpublished data). Antigen-positive peaks for each virus were pooled and dialyzed against PBS, and the volume was reduced by addition of Ficoll (Pharmacia). Continuous CsCl gradients (12.5 ml) from densities of 1.05 to 1.30 g/ml were poured, and

0.5-ml volumes of concentrated samples were loaded on top of each. Rate zonal centrifugation was carried out at 34,000 rpm for 2 h at 10°C in an SW40 rotor. Fractions were again tested in the appropriate enzyme-linked immunosorbent assay. After dialysis into PBS (pH 7.5), the antigens were again centrifuged to equilibrium in discontinuous CsCl density equilibrium gradients exactly as described above. Gradient fractions were tested for core antigen by enzyme-linked immunosorbent assays, using immunoglobulin G fractions of sera from HBcAg- or GSHcAg-positive carriers to coat flat-bottom microtiter plates (Dynatech) and for conjugation with horseradish peroxidase as described (3, 20). Fraction densities were derived from refractive index determinations, and absorbancy profiles were determined by measurements at 280 nm on a Zeiss PM2 spectrophotometer (10). DNA polymerase assays were carried out as described (1). For SDS-PAGE and peptide mapping, samples of core particles were dialyzed against 0.01 M sodium borate buffer (pH 8.5), containing 0.1% SDS, overnight at room temperature. Samples were then lyophilized and labeled with the Bolton-Hunter reagent (8, 10, 13). SDS-

FIG. 1. SDS-PAGE of radioacylated HBcAg and GSHcAg polypeptides. (a) GSHcAg. Arrows point to polypeptides 37,500, 26,000, and 20,500 daltons in size. (b) HBcAg. Arrows point to polypeptides 40,000, 27,500, and 22,000 daltons in size. The larger and lowest pair of arrows point to the major polypeptide of each core particle. (c) HBcAg. This polypeptide profile demonstrates the presence of higher-molecular-weight components in HBcAg and the cleaved polypeptide (arrow pointing to lowest-molecular-weight band) seen in some gels. The arrows here indicate all of the HBcAg-associated polypeptides that were subjected to tryptic peptide mapping.

FIG. 2. Tryptic peptide maps of the major polypeptides of HBcAg and GSHcAg. (a) HBcAg p22; (b) GSHcAg p20.5; (c) composite of p22 and p20.5 on the tracing of p22; (d) composite of p22 and p20.5 on the tracing of p20.5. In all peptide maps, electrophoresis in the first dimension was carried out from right to left, and chromatography in the second dimension was performed from bottom to top. Blackened spots (●) indicate spots shared by both polypeptides, open spots (○) indicate those spots unique to HBcAg p22 in (c) or those unique to GSHcAg p20.5 in (d), and the dashed spots (⊖) indicate the spots unique to the heterologous polypeptide in each composite (except this figure, where they are not shown). Composite peptide maps presented in the other figures can be analogously interpreted. Composites were constructed by overlaying the various autoradiograms in question, by overlaying the autoradiogram of a single polypeptide digest over the tracing of another, and by mixing experiments in which equal samples of the digests from two polypeptides were separated on the same plate. In most cases, multiple peptide maps and appropriate mixing experiments were carried out with each polypeptide and polypeptide pair to ascertain the percentage of homology.

PAGE was carried out on 12.5% separating slab gels in the discontinuous system of Laemmli (17) with several modifications (8, 13). Details of tryptic peptide mapping have already been described (10, 13).

RESULTS

SDS-gel profiles of HBcAg and GSHcAg polypeptides. Radioacylated core polypeptides were reduced, alkylated, and analyzed by SDS-PAGE as shown in Fig. 1. The major GSHcAg polypeptide moved slightly faster than the corresponding major HBcAg polypeptide. Similar analysis

of the core polypeptides which were phosphorylated by the virus-associated protein kinase reaction (1, 9) or stained by Coomassie blue (data not shown) also revealed a major GSHcAg polypeptide migrating slightly faster than the corresponding major HBcAg polypeptide. In addition, a number of HBcAg polypeptides both larger and smaller than the major component ran on the gel with mobilities similar to those seen with several GSHcAg polypeptides, suggesting further similarities among these core antigen particles. In these cases, polypeptide bands of

GSHcAg (p37.5, p26, and p20.5) migrated slightly faster on the gel than "analogous" HBcAg-associated species (p40, p27.5, and p22). The reproducible differences shown here by SDS-PAGE analysis of HBcAg and GSHcAg polypeptides, and the apparent size differences observed in the surface antigen polypeptides of the two viruses (8, 13), may point to differences in gene expression or the genetic organization in these two viruses, or both. To discern the nature of the differences, tryptic peptide mapping of the core polypeptides was undertaken.

Tryptic peptide mapping of the major polypeptides of HBcAg and GSHcAg. The major core polypeptides of HBV (p22) and GSHV (p20.5) were characterized by two-dimensional tryptic peptide mapping. The autoradiograms and composite drawings shown in Fig. 2, as well as mixing experiments (data not shown), revealed that 56% of the spots were shared by the two polypeptides. Results obtained from a second infected human liver and a second ground squirrel liver were the same in both polypeptide profile and peptide mapping. Despite the observation here that the major polypeptides of the core antigen particles from each virus shared a significant amount of homology, attempts to demonstrate serological cross-reactivity by using intact core particles and a variety of methods have only met with partial success. In addition, it appears that the major core antigen polypeptides of these viruses share considerably more primary structure than their corresponding surface antigen polypeptides (8, 13), perhaps reflecting different immunological pressures upon each of these gene products during evolution. Furthermore, it is interesting that although these major polypeptides show at least some differences in 44% of their primary sequence, most of the major sites of phosphorylation in each case are conserved, in that only selected amino acid residues within shared primary sequences are phosphorylated (10).

Relationship between the low-molecular-weight polypeptides and the major core polypeptide. Recently, Albin and Robinson (1) have described a cleavage of the major core polypeptide of HBcAg after incubation of core particles with anti-core-containing sera. We thus studied the tryptic peptide maps of core-associated polypeptides smaller than the major core component and looked for homology with the latter. The p19 of HBcAg and p16.5 of GSHcAg (Fig. 1 and 3) were identified as such components, and their peptide maps were compared with those of the appropriate major polypeptide (Fig. 4). In both cases, the number of spots seen in the lower-molecular-weight pair of bands was significantly less than that seen in the corresponding major core polypeptides. p19 of HBcAg contained only

37% of the spots seen in p22, and p16.5 of GSHcAg retained only 55% of the spots seen in p20.5. In HBcAg, this cleavage seemed to take place so that most of the positively charged peptides of p22 were absent from p19. This

HB _c Ag	GSH _c Ag
100*	
100*	
100*	
82	
65*	
52	54
	48
40	37.5
	34
31*	
27.5	26
<u>22</u>	<u>20.5</u>
19*	
	18
	16.5
14.5*	14.5
	12.5*

FIG. 3. Molecular weights of human and ground squirrel hepatitis virus core polypeptides. Polypeptides were labeled by radioacylation using the Bolton-Hunter reagent, reduced and alkylated, and analyzed by SDS-PAGE. Values are reported in daltons $\times 10^3$. Underscore designates the major labeled polypeptide. An asterisk next to polypeptide size designations indicates those not observed on every gel.

FIG. 4. Relationship between the low-molecular-weight polypeptides (HBcAg p19 and GSHcAg p16.5) and their major core polypeptides. (a) HBcAg p19; (b) GSHcAg p16.5; (c) composite of HBcAg p19 and p22 on a tracing of the latter; (d) composite of GSHcAg p16.5 and p20.5 on a tracing of the latter.

result is compatible with the suggestion (29) that the carboxy-terminal portion of the proposed HBcAg polypeptide sequence is highly basic in nature and suggests that p19 is the amino-terminal portion of p22. The point of cleavage of p20.5 of GSHcAg giving rise to p16.5 is probably different from that in HBcAg since the percentage of spots remaining in p16.5 was 55% whereas that in p19 of HBcAg was only 37%. Furthermore, a number of basic tryptic peptides shared between p20.5 of GSHcAg and p22 of HBcAg are retained after cleavage of the major ground squirrel core antigen polypeptide (Fig. 4). Tryptic peptide mapping of the other low-molecular-weight. These polypeptides retained most of the basic residues missing in p19 and probably represent either specific cleavage or breakdown products of p22 or higher-molecular-weight

core-associated polypeptides or both. The trend toward simpler peptide maps was also observed with low-molecular-weight GSHcAg polypeptides, and in some cases a subset of the spots within these maps was also seen in those of the core-associated polypeptides larger than the major component (data not shown). For example, p18 of GSHcAg had virtually the same peptide map as p34 in the gel profile, indicating dimerization of this minor low-molecular-weight polypeptide. Similarly, a possible explanation for the inconsistent appearance of HBcAg p31 may be that it is due to variable dimerization of p14.5. The small amounts of HBcAg p31 present on gels, however, did not permit peptide mapping to test this relationship.

Relationship between the high-molecular-weight polypeptides and the major core polypep-

FIG. 5. Relationship between the major high-molecular-weight polypeptides (HBcAg p40 and GSHcAg p37.5) and their major core polypeptides. (a) HBcAg p40; (b) GSHcAg p37.5; (c) composite of HBcAg p40 and p22 on the tracing of the latter; (d) composite of GSHcAg p37.5 and p20.5 on the tracing of the latter.

tide. Tryptic peptide mapping of high-molecular-weight core antigen-associated polypeptides was compared with the map of the major core polypeptide component of each virus (Fig. 5 and 6). All of the high-molecular-weight bands, like the low-molecular-weight components described above, shared amino acid sequences with their respective major core polypeptides (Fig. 5 and 6). Discrete polypeptide bands attributable to copurifying liver contaminants were not discerned at the level of peptide mapping within the limits of detection. Peptide mapping of radioacetylated human immunoglobulin heavy and light chains, often seen copurifying with core antigen particles, showed no homology with the maps derived from any of the core antigen-associated proteins (data not shown), even though there

were minor bands at molecular weight values close to those of immunoglobulin heavy and light chains in both GSHcAg and HBcAg preparations (Fig. 1 and 3). Furthermore, the specific relationship between a number of the large, minor polypeptide species and the major core component was inconsistent with that of simple aggregation, nor was the appearance of these high-molecular-weight species the result of disulfide bond exchange during electrophoresis, since the proteins were stably alkylated after reduction with iodoacetamide. All of the high-molecular-weight polypeptides from each virus shared a significant number of spots not observed in the maps of the major core polypeptide of the same virus (Fig. 5 and 6). Approximately two-thirds of the spots in the peptide maps of the

FIG. 6. Relationship between HBcAg p27.5 and GSHcAg p26 with their respective higher-molecular-weight polypeptides. (a) HBcAg p27.5; (b) GSHcAg p26; (c) composite of HBcAg p27.5 and p40 on the tracing of p27.5; (d) composite of GSHcAg p26 and p37.5 on the tracing of p26.

p27.5 band of HBcAg and p26 band of GSHcAg were shared with the respective major core polypeptide (data within Fig. 2 and 6). The p40 band of HBcAg and the p37.5 band of GSHcAg (often the most dominant of the minor high-molecular-weight bands; Fig. 1) showed that only 58 and 60% of the spots on their respective peptide maps were shared with the peptide map of the major core component in each case (Fig. 5). The number of unique spots seen in p27.5 of HBcAg and p26 of GSHcAg in comparison to their respective major polypeptides was a subset of the unique spots seen in the maps of HBcAg p40 and GSHcAg p37.5 when the same comparisons were made. This strongly suggests that

p37.5 of GSHcAg and p40 of HBcAg are not simple dimers of the major core component and that they may have significant additional primary sequence accounting for the difference in molecular weight values between these polypeptides and the major component. When p40 of HBcAg was compared with p27.5, 63% of the spots in p40 were found in the map of p27.5. In contrast, only 16% of the spots in p27.5 were not found in p40. Since different labelings and separations on two-dimensional thin layers result in an average of 8% variation in the spots for any given peptide map (8), p27.5 would appear to contain little or no primary sequence not also found in p40. Similarly, 66% of the spots of

GSHcAg p37.5 were shared with p26, whereas the percentage of spots unique to p26 was only 15%.

A p62-65 band appeared variably on several gels, and SDS-PAGE results suggest that this polypeptide may be a dimer of HBcAg p31. This polypeptide (p65) shared 75% homology with p40 and 63% homology with the major polypeptide of HBcAg (p22). However, when the map of p65 was compared with that of the p22 plus p40 composite, only 16% of the p65 spots were unique, suggesting that this polypeptide was a complex of p22 and p40. Furthermore, since the percentage of homology between p65 and p22 was the same as that reported for p40 and p22, it is likely that the increase in size from 40,000 daltons was not accompanied by any substantial increase in primary structure, again suggesting that p65 may be a complex of one or more polypeptides.

GSHcAg minor polypeptide bands at 48,000 and 56,000 daltons resulted in peptide maps sharing 80 to 90% homology with p37.5. This very close relatedness suggests that these increased molecular weight increments are not accompanied by an increase in primary sequence, and that these components may result from the aggregation of one or more lower-molecular-weight polypeptides. A similar result was seen with the p52 band of HBcAg. This band shared better than 75% homology with p37.5, whereas the map of p27.5 shared 84% homology with p52. Considering that much larger differences in the structure of polypeptides much closer in molecular weight have already been described, the differences in molecular weight between p27.5 and p52 do not suggest that p52 is unique. Similarly, p82 of HBcAg shared 94% homology with p40, suggesting dimerization in this case as well. Variable numbers of minor high-molecular-weight components greater than 100,000 daltons in size were seen on many gels after analysis of HBcAg polypeptides and on several gels with GSHcAg profiles. The peptide maps of these components similarly showed little increase in primary sequences that would account for their high molecular weight, suggesting that these are stable, higher aggregates of one or more proteins.

In summary, substantial increases in unique primary sequences compared to the major core polypeptide of each virus were obtained with increasing molecular weight of associated core components. The major high-molecular-weight polypeptides of HBcAg (p40) and GSHcAg (p37.5) demonstrated the greatest amount of additional unique sequences. Higher-molecular-weight polypeptides associated with each core preparation consistently showed few spots considered to be unique as compared to p40 in

HBcAg, p37.5 in GSHcAg, or the appropriate major core component. For a protein contaminant to account for these extra spots, it would have to migrate at multiple molecular weight values and bind tightly to the small quantities of minor polypeptide components and not at all with the major core polypeptide, even though the major polypeptide and high-molecular-weight minor components share 60% or more homology. It is suggested, then, that the p40 band of HBcAg and p37.5 band of GSHcAg represent the largest unique translation products in the core antigen particles of HBV and GSHV, respectively.

DISCUSSION

The development of the ground squirrel-GSHV system as a model for hepatitis B infection is, in part, dependent upon the detailed molecular characterization of the virus-associated polypeptides. In agreement with other studies (6, 15, 21), SDS-PAGE analysis of HBcAg polypeptides resulted in a single major component, p22, which had a mobility similar to that of the major core antigen polypeptide of GSHV (p20.5). Combined with the 56% homology seen in the tryptic peptide map composite of p22 and p20.5 (Fig. 2), these results suggest that the amount and sequence of DNA encoding these polypeptides are similar in the two viruses.

Tryptic peptide mapping of the major polypeptide and often the most intense of the smaller polypeptides on gels identified p19 in HBcAg and p16.5 in GSHcAg as the most likely candidates resulting from partial cleavage of the major core polypeptide of each virus. It should be noted that this cleavage product is in the same molecular weight range as e antigen isolated as a free polypeptide from serum (19, 27, 28). Other studies examining the nature of e antigen in Dane particle cores reveal activity in a 45,000-dalton band as well as in the major polypeptide, p19 (27). When these core particles were treated with pronase, the 45,000-dalton band yielded bands 31,000 and 15,000 daltons in size. Further heating of this 31,000-dalton band resulted in gels showing only the 15,000-dalton band, suggesting that the 45,000- and 31,000-dalton polypeptides were aggregates of the basic e antigen component. Although p31 of HBcAg was not mapped due to technical considerations, a 14,500-dalton band was present on most HBcAg gels, which may have been its monomeric form and may explain the infrequent appearance of p31. The GSHcAg polypeptide analogous to p31 of HBcAg was p34, and this turned out to be a dimer of GSHcAg p18 at the level of peptide mapping. Since p19 and p14.5 of HBcAg share considerable primary sequence (data not shown) and p14.5 seems to be the smallest polypeptide

with e antigen activity thus far reported, it is possible that the action of cleavage in one or more of the core antigen components may result in the generation of low-molecular-weight e antigen-positive polypeptides. The cleavage of core polypeptides may be important in uncoating the nucleoprotein complex within, after infection of hepatocytes with HBV or GSHV.

The nature of at least some of the high-molecular-weight core-associated polypeptides observed in this study and in others (6, 15, 21) was resolved by peptide mapping. A pair of studies analyzing the polypeptides of liver- (6, 15) or Dane particle-derived core particles (12, 15) consistently found a major high-molecular-weight band at 35,000 or 37,000 daltons, respectively. The results herein showed a polypeptide band which was dominant among the high-molecular-weight group at 40,000 daltons for HBcAg and at 37,500 daltons for GSHcAg (Fig. 1 and 3). In Dane particle cores, another polypeptide of 25,000 daltons was reported, which is probably analogous to the p27.5 band of HBcAg and the p26 band of GSHcAg. The large number of unique spots in p40 of HBcAg and p37.5 of GSHcAg (about 40% of the peptide maps of these components) is likely to be additional primary sequences and not contaminating host components because this single contaminating protein would have to comigrate with p40, p37.5, p27.5, and p26, even though these components are at different molecular weights. Furthermore, the 60% or better homology between p40 of HBcAg or p37.5 of GSHcAg and their respective major polypeptides would make it unlikely that a contaminant would bind so tightly to these minor components and not at all to the much larger quantities of the major core polypeptide in each gel. It is proposed, therefore, that the genome of HBV and GSHV encodes for these larger polypeptides and that they are integral core components. This would be accomplished by translation of the open reading frame on the long strand preceding the proposed N-terminal amino acid of p22 HBcAg. This open reading frame is 462 base pairs in length, transverse the nick in the long strand, and terminates approximately 20 base pairs from the 3' end of the nick of this same strand (29). The region would encode 154 amino acids. If a transcript made from this region were spliced to the proposed open reading frame encoding the major core polypeptide, a polypeptide approximately 40,000 daltons in size and containing 360 amino acids would be made. Since a TATA-like sequence for RNA polymerase binding is not detected between the amino terminus of this large core polypeptide (p40) and the stop codon of the major surface antigen polypeptide gene, it is possible that core and surface antigen poly-

peptide-encoding transcripts may share the same promoter region located upstream from the first ATG codon of the pre-HBs gene sequence. About 280 base pairs downstream from the N-terminus of the 40,000-dalton polypeptide is another TATA-like sequence (11, 29). If another mRNA is generated near this point and continues as described above to the proposed carboxy terminus of the major core polypeptide before termination of translation occurs, then a polypeptide with a molecular weight of 27,000 will be made. This polypeptide will share considerable structural homology with the major core polypeptide and have a number of unique spots which are shared with p40 of HBcAg. The finding of these sizes and number of polypeptides in both the HBcAg and GSHcAg profiles, and no other bands, is consistent with this type of proposal for the genetic organization of core polypeptides in both viruses. Although other open reading frames exist on the short strand of the HBV genome for translation, polypeptides resulting from the translation of each segment, fused to sequences of the major core polypeptide, would require transcription of a single messenger from both DNA strands. Since the high-molecular-weight polypeptides in each core antigen preparation showed little further unique information, and since most of them were identified as aggregates of lower-molecular-weight components, it is unlikely that larger unique core antigen polypeptides exist. The genetic organizations of the HBV and GSHV core antigen genes, therefore, seem to be similar.

ACKNOWLEDGMENTS

We thank Susan Knight for excellent technical assistance. This study was supported by Public Health Service research grant AI-13526 from the National Institutes of Health and by American Cancer Society research grant MV 95. M.A.F. was supported by an individual postdoctoral fellowship (grant PF-1792) from the American Cancer Society.

LITERATURE CITED

1. Albin, C., and W. S. Robinson. 1980. Protein kinase activity in hepatitis B virus. *J. Virol.* 34:297-302.
2. Almeida, J. D., D. Rubenstein, and E. J. Stott. 1971. New antigen-antibody system in Australia-antigen-positive hepatitis. *Lancet* ii:869-873.
3. Avrameas, S., and T. Terynck. 1971. Peroxidase-labeled antibody and Fab conjugates with enhanced intracellular penetration. *Immunochemistry* 8:1175-1179.
4. Barker, L. F., J. D. Almeida, J. H. Hoofnagle, R. J. Gerety, R. D. Jackson, and P. P. McGrath. 1974. Hepatitis B core antigen: immunology and electron microscopy. *J. Virol.* 14:1552-1558.
5. Budkowska, A., B. Kalinowska, and A. Nowoslawski. 1979. Identification of two HBcAg subspecificities revealed by chemical treatment and enzymatic digestion of liver derived HBcAg. *J. Immunol.* 123:1415-1416.
6. Budkowska, A., J. W.-K. Shih, and J. L. Gerin. 1977. Immunochemistry and polypeptide composition of hepatitis B core antigen (HBcAg). *J. Immunol.* 118:1300-1305.
7. Dane, D. S., C. H. Cameron, and M. Briggs. 1970. Virus-like particles in serum of patients with Australia antigen

- associated hepatitis. *Lancet* ii:695-698.
8. Feitelson, M. A., P. L. Marion, and W. S. Robinson. 1981. Antigenic and structural relationships of the surface antigens of hepatitis B virus, ground squirrel hepatitis virus, and woodchuck hepatitis virus. *J. Virol.* 39:447-454.
 9. Feitelson, M. A., P. L. Marion, and W. S. Robinson. 1982. Core particles of hepatitis B virus and ground squirrel hepatitis virus. II. Characterization of the protein kinase reaction associated with ground squirrel hepatitis virus and hepatitis B virus. *J. Virol.* 43:741-748.
 10. Feitelson, M. A., F. O. Wettstein, and J. G. Stevens. 1981. Tryptic peptide mapping of picomolar quantities of protein labeled with the Bolton-Hunter reagent. *Anal. Biochem.* 116:473-479.
 11. Galibert, F., E. Mandart, F. Fitoussi, P. Tiollais, and P. Charnay. 1979. Nucleotide sequence of the hepatitis B virus genome (subtype ayw) cloned in *E. coli*. *Nature (London)* 281:646-650.
 12. Gerin, J. L., and W. K. Shih. 1978. Structure of HBsAg and HBcAg, p. 147-158. *In* G. N. Vyas, S. N. Cohen, and R. Schmidt (ed.), *Viral hepatitis*. Franklin Institute Press, Philadelphia.
 13. Gerlich, W. H., M. A. Feitelson, P. L. Marion, and W. S. Robinson. 1980. Structural relationships between the surface antigens of ground squirrel hepatitis virus and human hepatitis B virus. *J. Virol.* 36:787-795.
 14. Hoofnagle, J. H., R. J. Gerety, and L. F. Barker. 1973. Antibody to hepatitis B virus core in man. *Lancet* ii:869-873.
 15. Hruska, J. F., and W. S. Robinson. 1977. The proteins of hepatitis B Dane particle cores. *J. Med. Virol.* 1:119-131.
 16. Kaplan, P. M., R. L. Greenman, J. L. Gerin, R. H. Purcell, and W. S. Robinson. 1973. DNA polymerase associated with human hepatitis B antigen. *J. Virol.* 12:995-1005.
 17. Laemmli, U. K. 1970. Cleavage of structural proteins during assembly of the head of bacteriophage T₄. *Nature (London)* 227:680-685.
 18. Marion, P. L., L. S. Oshiro, D. C. Regnery, G. H. Scullard, and W. S. Robinson. 1980. A virus in Beechey ground squirrels that is related to hepatitis B virus of humans. *Proc. Natl. Acad. Sci. U.S.A.* 77:2941-2945.
 19. McAuliffe, V. J., and R. H. Purcell. 1978. Current status of tests for HBeAg and anti-HBe, p. 161-171. *In* G. N. Vyas, S. N. Cohen, and R. Schmidt (ed.), *Viral hepatitis*. Franklin Institute Press, Philadelphia.
 20. Nakane, P. K., and A. Kawaoi. 1974. Peroxidase-labeled antibody. A new method of conjugation. *J. Histochem. Cytochem.* 22:1084-1091.
 21. Neurath, A. R., S.-N. Huang, and N. Strick. 1978. Some properties of hepatitis B core antigen isolated from serum of infected humans. *J. Gen. Virol.* 39:91-101.
 22. Neurath, A. R., and N. Strick. 1979. Association of hepatitis B e-antigen (HBeAg) determinants with the core of Dane particles. *J. Gen. Virol.* 42:645-649.
 23. Ohoi, H., S. Onodera, and N. Ishida. 1979. Demonstration of hepatitis B e antigen (HBeAg) in association with intact Dane particles. *J. Gen. Virol.* 43:423-427.
 24. Robinson, W. S., D. A. Clayton, and R. L. Greenman. 1974. DNA of a human hepatitis B virus candidate. *J. Virol.* 14:384-391.
 25. Robinson, W. S., and R. L. Greenman. 1974. DNA polymerase in the core of the human hepatitis B virus candidate. *J. Virol.* 13:1231-1236.
 26. Takahashi, K., Y. Akahane, T. Gotanda, T. Mishiro, M. Imai, Y. Miyakawa, and M. Mayumi. 1979. Demonstration of hepatitis e antigen in the core of Dane particles. *J. Immunol.* 122:275-279.
 27. Takahashi, K., M. Imai, T. Gotanda, T. Sano, A. Oinuma, S. Mishiro, Y. Miyakawa, and M. Mayumi. 1980. Hepatitis B e antigen polypeptides isolated from sera of individuals infected with hepatitis B virus: comparison with HBeAg polypeptide derived from Dane particles. *J. Gen. Virol.* 50:49-57.
 28. Tedder, R. S., and F. G. Bull. 1979. Characterization of "e" antigen associated with hepatitis B. *Clin. Exp. Immunol.* 35:380-389.
 29. Tiollais, P., P. Charnay, and G. N. Vyas. 1981. Biology of hepatitis B virus. *Science* 213:406-411.
 30. Yoshizawa, H., Y. Itoh, J. P. Simonetti, T. Takahashi, A. Machida, Y. Miyakawa, and M. Mayumi. 1979. Demonstration of hepatitis B e antigen in hepatitis B core particles obtained from the nucleus of hepatocytes infected with hepatitis B virus. *J. Gen. Virol.* 42:513-519.