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Proteins of Hepatitis B Surface Antigen: Amino Acid Compositions of the Major Polypeptides

J. WAI-KUO SHIH AND JOHN L. GERIN*
Molecular Anatomy Program, Oak Ridge National Laboratory, Rockville, Maryland 20852

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The major polypeptides isolated from two different preparations of hepatitis B surface antigen/*adw* were analyzed for their amino acid compositions. The results indicated a high degree of compositional relatedness between the P-1 (23,000, molecular weight) and P-2 (29,500, molecular weight) polypeptides for each of the two preparations. A considerable proportion of the major P-1 and P-2 polypeptides may be composed of a common structure. No amino sugars were detected in the preparations of isolated polypeptides.

The approximately 22-nm form of hepatitis B surface antigen (HB_sAg) consists of at least seven polypeptides of which P-1 (23,000 daltons) and P-2 (29,500 daltons) are the major components (3, 13, 14). A third polypeptide, P-6 (72,000 daltons), is observed as a major polypeptide in some preparations. All of the polypeptides appear to contain the recognized group (*a*) and subtype (*d* or *y*) virus-specific determinants as part of their constituent structures (6, 13). The P-2 component may be a glycoprotein on the basis of its reaction with periodic acid-Schiff reagent in sodium dodecyl sulfate (SDS)-polyacrylamide gels (14). We report here a comparison of the major polypeptides by amino acid compositional analysis.

The 22-nm forms of HB_sAg were purified from the plasma of HB_sAg carriers by zonal centrifuge techniques (2, 4, 5). The individual polypeptides were isolated by SDS-polyacrylamide gel electrophoresis as previously described (13). Polymerized gels contained 5% acrylamide, 0.125% (wt/vol) *N-N'*-methylenebisacrylamide, 0.1% (wt/vol) ammonium persulfate, 0.05% TEMED (*N,N,N',N*-tetramethylenediamine), and 0.1% (wt/vol) SDS in 0.1 M sodium phosphate buffer (pH 7.2) and were subjected to preelectrophoresis for 30 min before use. Preparations of purified HB_sAg were solubilized by incubation with 1% (wt/vol) SDS, 1% (vol/vol) 2-mercaptoethanol, 10% (vol/vol) glycerol, and 0.02% (wt/vol) bromophenol blue at 37°C for 2 h followed by 60°C for 10 min. A sample of approximately 250 μg of Lowry protein was applied to each preparative gel (9 by 100 mm) which was then subjected to electrophoresis for 10 h at 15 mA/gel. The regions of the gel corresponding to the individual polypeptides were located by comparison with a marker gel stained for protein with Coomassie blue (15); these regions were separately excised and extracted twice with a solution of 0.05% (wt/vol) SDS and 0.005 M $NaHCO_3$ (15). The final extracts were lyophilized, and the dry residues were dissolved in water and stored at −70°C. The polypeptides of two HB_sAg/*adw* preparations (630198 and DRAA) were isolated by these procedures. Of the seven HB_sAg polypeptides, only P-1, P-2, P-6, and P-7 were isolated. No attempt was made to isolate the P-3, P-4, or P-5 polypeptides since they were either poorly separated or low in concentration.

The purity of the isolated polypeptides was assessed by reelectrophoresis in 7.5% SDS-polyacrylamide gels. For the 630198 preparation (Fig. 1), the polypeptides migrated to their characteristic positions when compared with the native antigen (Fig. 1A). The P-1, P-2, and P-7 preparations were free of detectable contamination with other polypeptides, whereas the P-6 material contained a small quantity of P-7. An analysis of the DRAA preparation yielded similar results as previously described (13). The major polypeptides (P-1, P-2, and P-6) of the two HB_sAg/*adw* preparations were analyzed for their amino acid compositions using a Durrum D-500 analyzer equipped with a single column-three buffer system. Samples were hydrolyzed in 6 M HCl at 105° for 20 h in evacuated and sealed tubes with norleucine added as an internal standard for calibration of recovery. Hydrolysates of the 630198 polypeptides (P-1, P-2, and P-6) were each analyzed three times with essentially identical results, and the calculated values represent an average of these analyses. The DRAA P-6 hydrolysate was analyzed twice and the DRAA P-1 and P-2 hydrolysates were analyzed once, due to limited quantities of

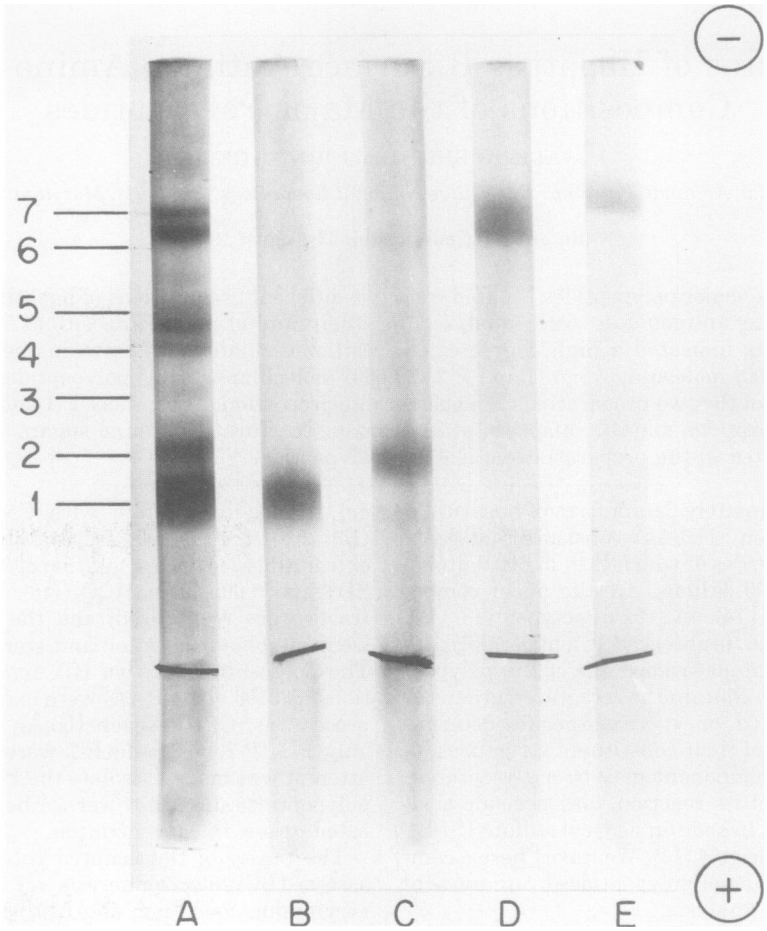

FIG. 1. *Polyacrylamide gel electrophoresis of the isolated polypeptides of 630198 HB_sAg. The native 630198 preparation (15.6 μg) was solubilized in 1% SDS and 1% 2-mercaptoethanol at 90°C for 2 min before electrophoresis on 7.5% SDS-polyacrylamide gels for 4 h at 8 mA/gel (A). Gels containing the isolated polypeptides were run in parallel: (B) P-1 (6.4 μg), (C) P-2 (3.1 μg), (D) P-6 (2.9 μg), and (E) P-7 (2.0 μg). The gels were stained for protein with Coomassie blue by the method of Weber and Osborn (15) and photographed.*

these polypeptide samples. A duplicate sample of each of the six polypeptide preparations were oxidized in performic acid for 4 h before acid hydrolysis for determination of their cystine and methionine contents (10).

The amino acid compositions of the major P-1 and P-2 polypeptides for each of the two HB_sAg/adw preparations are shown in Table 1. The major polypeptides were compared using the method of Metzger et al. (9) to assess the relatedness of two different proteins. Difference indexes (DI) were calculated by summing the differences in the fractional contents of each amino acid and multiplying the sum by 50. DI values of 2.7 and 6.1 for the P-1 versus P-2 compositions of 630198 and DRAA, respectively, indicated a close compositional relatedness between these two major polypeptides. Similarly, a high degree of relatedness was observed when DRAA P-1 was compared with 630198 P-1 (DI = 7.1) and DRAA P-2 with 630198 P-2 (DI = 6.6). Comparison of the amino acid compositions of the P-6 components of DRAA and 630198 (Table 2) revealed a lower but significant degree of relatedness (DI = 11.4). Interestingly, the P-6 component of DRAA appears to differ significantly in its composition from that of the DRAA P-2 polypeptide (DI = 22.4); the P-6 and P-2 components of 630198 were more closely associated (DI = 14.9). This appeared to correlate with the higher proportion of DRAA P-6 (13) than 630198 P-6 (Fig. 1) in the native HB_sAg preparations.

The P-1 and P-2 polypeptides differ in their

TABLE 1. *Amino acid composition of HB_sAg polypeptides P-1 and P-2*

Amino acid	DRAA			630198		
	Mole fraction		$\|\Delta\|^a$	Mole fraction		$\|\Delta\|^b$
	P-1	P-2		P-1	P-2	
Aspartic acid	0.072	0.070	0.002	0.056	0.053	0.003
Threonine	0.081	0.081	0.000	0.096	0.097	0.001
Serine	0.130	0.126	0.004	0.125	0.125	0.000
Glutamic acid	0.085	0.057	0.028	0.050	0.043	0.007
Proline	0.107	0.104	0.003	0.117	0.114	0.003
Glycine	0.088	0.091	0.003	0.099	0.091	0.008
Alanine	0.046	0.041	0.005	0.040	0.039	0.001
Half-cystine	0.057	0.098	0.041	0.058	0.064	0.006
Valine	0.044	0.048	0.004	0.047	0.048	0.001
Methionine	0.020	0.020	0.000	0.029	0.023	0.006
Isoleucine	0.046	0.049	0.003	0.052	0.058	0.006
Leucine	0.122	0.117	0.005	0.120	0.120	0.000
Tyrosine	0.009	0.006	0.003	0.011	0.015	0.004
Phenylalanine	0.043	0.053	0.010	0.052	0.055	0.003
Histidine	0.005	0.004	0.001	0.008	0.010	0.002
Lysine	0.024	0.017	0.007	0.019	0.021	0.002
Arginine	0.024	0.021	0.003	0.022	0.023	0.001
Total			0.122			0.054
Difference index			6.10			2.70

[a] Difference between P-1 and P-2 of DRAA preparation.
[b] Difference between P-1 and P-2 of 630198 preparation.

TABLE 2. *Amino acid composition of HB_sAG polypeptide P-6*

Amino acid	Mole fraction		$\|\Delta\|^a$
	DRAA (P-6)	630198 (P-6)	
Aspartic acid	0.092	0.081	0.011
Threonine	0.056	0.073	0.017
Serine	0.084	0.117	0.033
Glutamic acid	0.116	0.087	0.029
Proline	0.088	0.075	0.013
Glycine	0.063	0.105	0.042
Alanine	0.090	0.073	0.017
Half-cystine	0.043	0.042	0.001
Valine	0.056	0.056	0.000
Methionine	0.013	0.013	0.000
Isoleucine	0.017	0.033	0.016
Leucine	0.103	0.097	0.006
Tyrosine	ND[b]	0.013	ND
Phenylalanine	0.048	0.043	0.005
Histidine	0.028	0.013	0.015
Lysine	0.069	0.046	0.023
Arginine	0.031	0.031	0.000
Total			0.228
Difference index			11.40

[a] Difference between P-6 of DRAA and 630198 preparation.
[b] ND, Not determined; not included in difference index estimate.

apparent molecular weights by 6,500. Based on previous estimates for the carbohydrate content of HB_sAg (10) and the pattern of periodic acid-Schiff stain reaction in polyacrylamide gels (14), carbohydrate should comprise no more than 25% by weight of the P-2 molecule and would account for an overestimate in molecular weight of about 2,500 (12). Therefore, the P-1 and P-2 polypeptides actually differ in molecular weight by at least 4,000. Interestingly, no amino sugars were detected in any of the preparations of isolated polypeptides including the DRAA P-2 and 630198 P-2 fractions. It appears that if the P-2 polypeptide is indeed a glycoprotein, it does not contain glucosamine or galactosamine at this level of analysis.

The Dane particle, which probably represents the virion of hepatitis B virus, contains a double-stranded circular DNA with a molecular weight of 1.6×10^6 (11). A DNA of this size could code for approximately 80,000 daltons of primary gene product but must account for the antigenic determinants of HB_sAg, structural proteins of the core (HB_cAg), and, possibly, components of the *e*-Ag complex (8) and the DNA polymerase (7). HB_sAg consists of at least seven polypeptides, and the sum of their molecular weights alone exceeds this amount of available genetic information. However, all of the polypeptides contain both the group and subtype determinants as part of their structures and therefore possess some protein sequences in common. The close compositional relatedness of two of the major polypeptides (P-1 and P-2) as described in this report indicate that these common sequences may comprise a considerable

proportion of the polypeptide structure. The P-6 components were less closely related to the P-1 and P-2 polypeptides, however, the proportion of P-6 in native HB_sAg varied between preparations as did the DI values. In addition to the P-6 component, the P-6 isolates may contain varying amounts of another protein of this molecular weight and not specified by the virus, such as human serum albumin. Common sequences among the HB_sAg polypeptides would suggest that hepatitis B virus replication in the human host may involve such mechanisms as posttranscriptional or posttranslational cleavage to make efficient use of limited genetic information.

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LITERATURE CITED

1. **Chairez, R., S. Steiner, J. L. Melnick, and G. R. Dreesman.** 1973. Glycoproteins associated with hepatitis B antigen. Intervirology 1:224–228.
2. **Gerin, J. L.** 1972. Isolation and physicochemical characteristics of HB Ag, p. 205–219. *In* G. N. Vyas and H. A. Perkins (ed.), Hepatitis and blood transfusion. Grune and Stratton, New York.
3. **Gerin, J. L.** 1974. Structure of hepatitis B antigen (HB Ag), p. 215–223. *In* W. S. Robinson and C. F. Fox (ed.), Mechanisms of virusdisease. W. A. Benjamin Inc., Menlo Park.
4. **Gerin, J. L., R. M. Faust, and P. V. Holland.** 1975. Biophysical characterization of the *adr* subtype of hepatitis B antigen and preparation of anti-*r* sera in rabbits. J. Immunol. 115:100–105.
5. **Gerin, J. L., P. V. Holland, and R. H. Purcell.** 1971. Australia antigen: large-scale purification from human serum and biochemical studies of its proteins. J. Virol. 7:569–576.
6. **Gold, J. W. M., J. W-K. Shih, R. H. Purcell, and J. L. Gerin.** 1976. Characterization of antibodies to the structural polypeptides of HB_sAg: evidence for subtype-specific determinants. J. Immunol. 117:1404–1406.
7. **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.
8. **Magnius, L. O., and J. Å. Espmark.** 1972. New specificities in Australia Antigen-positive sera distinct from the Le Bouvier determinants. J. Immunol. 109:1017–1021.
9. **Metzger, H., M. B. Shapiro, J. E. Mosimann, and J. E. Vinton.** 1968. Assessment of compositional relatedness between proteins. Nature (London) 219:1166–1168.
10. **Moore, S.** 1963. On the determination of cystine as cysteic acid. J. Biol. Chem. 238:235–237.
11. **Robinson, W. S., D. A. Clayton, and R. L. Greenman.** 1974. DNA of a human hepatitis B virus candidate. J. Virol. 14:384–391.
12. **Segrest, J. P., and R. L. Jackson.** 1972. Molecular weight determination of glycoproteins by polyacrylamide gel electrophoresis in sodium dodecyl sulfate, p. 54–63. *In* V. Ginsburg (ed.), Methods in enzymology, vol. 28. Academic Press Inc., New York.
13. **Shih, J. W-K., and J. L. Gerin.** 1975. Immunochemistry of hepatitis B surface antigen (HB_sAg): preparation and characterization of antibodies to the constituent polypeptides. J. Immunol. 115:634–639.
14. **Shih, J. W-K., and J. L. Gerin.** 1976. Proteins of hepatitis B surface antigen J. Virol. 21:347–357.
15. **Weber, K., and M. Osborn.** 1969. The reliability of molecular weight determinations by dodecyl sulfate-polyacrylamide gel electrophoresis. J. Biol. Chem. 244:4406–4412.