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Selection of peptide inhibitors of interactions involved in complex protein assemblies: Association of the core and surface antigens of hepatitis B virus

(phage display/peptide libraries/morphogenesis/antiviral agent)

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ABSTRACT **As an example for studies of contacts involved in complex biological systems, peptide ligands that bind to the core antigen of hepatitis B virus (HBcAg) have been selected from a random hexapeptide library displayed on filamentous phage. Affinity-purified phage bearing aa sequence LLGRMK, or some related sequences, bound full-length or truncated HBcAg but did not bind denatured HBcAg. The long (L), but not the short (S), hepatitis B virus envelope polypeptide, when synthesized in an *in vitro* system, bound firmly to HBcAg, indicating that interaction between HBcAg and the pre-S region of the L polypeptide is critical for virus morphogenesis. This interaction was inhibited by peptide ALLGRMKG, suggesting that this and related small molecules may inhibit viral assembly.**

Coliphage fd carrying random hexapeptide sequences in their gpIII protein have emerged as powerful tools for studies of ligand binding in the absence of structural or even sequence information (1–3). These phage have found use in a range of studies—including mapping the binding sites of antibodies (1, 3, 4), a chaperone protein (5), and cell-surface receptors (6). As a test system for the identification of contact regions between the components of complex biological structures such as organelles, viruses, or other multicomponent assemblies, we have used such fusion phage to explore interactions between the core and surface antigen components of hepatitis B virus (HBV). The results offer a guide to a critical stage of viral morphogenesis and approaches to its inhibition by small molecules, derived from short peptides, resembling the contact regions.

HBV consists of a nucleocapsid, the small 3.2-kb DNA genome, and the viral polymerase enclosed by the core antigen of the virus, surrounded in turn by the HBV viral surface antigen (HBsAg). The viral envelope contains three different, but related, HBsAg polypeptides, which overlap extensively from their carboxyl termini and arise from variable use of initiation triplets at different points within a continuous open reading frame. The long polypeptide (L polypeptide) is the product of the entire reading frame and comprises the pre-S1 domain of 108 amino acids (or 119, depending on virus subtype) at its amino terminus followed by the pre-S2 domain of 55 amino acids and the short polypeptide (S polypeptide) region of 226 amino acids. The medium-length polypeptide (M polypeptide) has the pre-S2 domain at its amino terminus followed by the S region, whereas the S polypeptide, which is the most abundant form, consists of only the S region. The pre-S regions are believed to play a role in both viral assembly (7, 8) and attachment to the host cell (9–11). The S form is more abundant than the M and L forms of HBsAg in the virus (12) and is also by far the most abundant form in the 22-nm particles of HBsAg that are found in great excess over the virus in the plasma of infected individuals.

The HBV core antigen (HBcAg) can be synthesized efficiently in *Escherichia coli* (13, 14), where it assembles to form 27-nm particles equivalent morphologically to those found in the liver of infected individuals (15). This provides a convenient source of HBcAg and derivatives of it for identification of peptide sequences carried by fusion phage that bind the antigen. The L and S HBsAg forms were synthesized in cell-free systems for analysis of their interaction with HBcAg and its inhibition by antibodies and peptides identified via the fusion phage libraries.

EXPERIMENTAL PROCEDURES

Materials. The hexapeptide fusion phage library (1) and *E. coli* strain K91Kan were from G. Smith (University of Missouri, Columbia). Monoclonal antibody 18/7 (purified IgG) was from K. H. Heermann and W. H. Gerlich (University of Göttingen, Göttingen, F.R.G.). Peptides ALLGRMKG, ALLTRILG, GRMKG, and LDPAFR were provided by R. Ramage (University of Edinburgh).

Plasmids pMDHBs3 and pMDHBs4, encoding the L and S forms of HBsAg, respectively, were used as templates for *in vitro* transcription reactions. Both contained a T7 RNA polymerase promoter followed by a 586-bp copy of the encephalomyocarditis virus RNA 5′ noncoding region (ref. 16; Novagen), 5′ to the HBsAg coding regions that were generated as *Eco*RI–*Sal* I DNA fragments from plasmid pHBV130 (17). PCR amplification was used to create *Eco*RI targets by double mutations at either $A^{922} \rightarrow G$ and $T^{923} \rightarrow A$ or $G^{1415} \rightarrow A$ and $G^{1419} \rightarrow C$ for the L- and S-coding fragments, respectively, and a downstream site for *Sal* I ($A^{2274} \rightarrow G$) common to both fragments.

Purification of Particles Comprising Full-Length or Truncated HBcAg. Purification of HBcAg from *E. coli* RB 791 harboring various plasmids (18, 19) was as described (20), except that the cell extract was precipitated by ammonium sulfate (35% saturation), dialyzed against TBS (50 mM Tris·HCl, pH 7.5/150 mM NaCl), applied to 8–40% sucrose gradients (12 ml; TBS), and centrifuged at 100,000 × *g* (TH641 rotor, Sorvall) for 5 hr at 4°C. Fractions containing HBcAg were pooled, and proteins were judged to be >90% pure by densitometry on SDS/PAGE, analytical sucrose-gradient centrifugation, and immune reactivity. Denaturation of truncated HBcAg was achieved by heating a sample (1

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Abbreviations: BSA, bovine serum albumin; HBV, hepatitis B virus; HBcAg, HBV core antigen; HBsAg, HBV surface antigen; pfu, plaque-forming units; L polypeptide, long polypeptide; S polypeptide, short polypeptide.

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mg/ml) to 85°C for 5 min, clarification by centrifugation, and remeasuring the soluble protein concentration.

Isolation of Phage that Bind to HBcAg. Nitrocellulose membrane (BioBlot-NC, 0.45-μM pore size, Costar) was soaked in 15% (vol/vol) methanol/25 mM Tris base/250 mM glycine for 15 min and placed on a dot-blot apparatus; HBcAg [20 μl; 0.25 mg/ml in phosphate-buffered saline (PBS)] was then washed through the membrane. The excised circles were placed in siliconized microcentrifuge tubes containing blocking buffer [400 μl; bovine serum albumin (BSA) at 10 mg/ml/0.5% Tween/0.02% NaN_3/TBS] and left at 6°C overnight. An aliquot of the phage library [5×10^{10} plaque-forming units (pfu)] was incubated with TBS (400 μl)/BSA (0.1 mg/ml)/Tween (0.5%) for 1 hr at 6°C, to absorb those phage that bind BSA. The discs were then placed in the same buffer containing the phage library and rotated at 6°C for 4 hr and washed six times with wash buffer A (0.5% Tween/TBS) or wash buffer B (0.5% Tween/50 mM Tris·HCl, pH 7.5/0.5 M NaCl), with a 10-min interval between each wash. Finally, elution buffer (400 μl; 0.1 M HCl, titrated to pH 2.2 by the addition of solid glycine/BSA at 1 mg/ml) was added, and after 10 min eluates were neutralized by addition of Tris·HCl (38 μl, 1 M, pH 9), titered, and amplified (21). Amplified eluates were subjected to two further rounds of affinity enrichment. DNA was isolated from individual phage clones (21), and the nucleotide sequence was determined (22) by using primer 5′-AGTTTT-GTCGTCTTTCC-3′. Selected phage plaques were amplified in 500-ml cultures and purified by PEG precipitation and equilibrium centrifugation in 31% (wt/wt) CsCl/TBS (21).

Phage-Binding Assay in Solution. HBcAg at various concentrations (0.3–10 μM) was incubated at 6°C for 18 hr with fusion phage B1 (10^9 pfu/ml) in TBS/BSA (0.2 mg/ml)/NaN_3 (0.02%). Aliquots (100 μl) of each mixture were transferred to polystyrene wells (no. 2585, Costar) that had been coated with HBcAg (20 μg/ml in PBS; 125 μl per well). After 1 hr at 6°C the wells were washed 10 times with TBS/BSA at 0.2 mg/ml. Bound phage were recovered and titered as described in the previous section. All assays were done in triplicate. The HBcAg concentration range was 1.58–50 μM for experiments with phage B2 and B3 and was 0.63–20 μM for experiments with phage B4. For peptide inhibition experiments, fusion phage (10^9 pfu/ml; 200 μl) were incubated with various concentrations of peptide (1 mM–10 nM) in HBcAg-coated wells for 90 min at 6°C.

***In Vitro* Transcription, Translation, and Translocation.** Templates for transcription were linearized by digestion with *Sal* I. Transcription reactions were done as described (23) by using T7 RNA polymerase (Promega). Synthetic RNAs were stored at −70°C in 4-μl aliquots. Translations were done at 30°C for 2 hr by using micrococcal nuclease-treated rabbit reticulocyte lysates (Flexi rabbit reticulocyte lysate system, Promega). Reactions (18 μl) contained 2 μl of a 1:10 dilution of the transcription reaction, 10 μl of rabbit reticulocyte lysate, 20 μM of amino acid mixture minus methionine, 0.7 μl of [^{35}S]methionine (1 Ci/mol, Amersham; 1 Ci = 37 GBq), 0.6 mM $Mg(OAc)_2$, 120 mM KCl, and 2 mM dithiothreitol. Reactions were done in the presence or absence of 0.1 μg of HBcAg and 1.3 μl of canine pancreatic microsomal membranes (2 equivalents/μl; Promega).

Immunoprecipitations. Translation mixture (5 μl) was diluted to 200 μl with NET-gel buffer (50 mM Tris·HCl, pH 7.5/150 mM NaCl/0.1% Nonidet P-40/1 mM EDTA/0.25% gelatin/0.02% NaN_3), containing 2 mM dithiothreitol. Either undiluted anti-HBsAg (a 1:1 mixture of anti-native and anti-denatured HBsAg sera) or a 1:10 dilution of anti-HBcAg rabbit polyclonal serum (1.5 μl) was added to the mixture. Immunoprecipitation with protein A-Sepharose and analysis by SDS/PAGE were as described (24).

Inhibition of Membrane-Inserted L-Protein Binding to HBcAg by Antibodies and Peptides. Canine pancreatic microsomal membranes containing ^{35}S-labeled L protein were purified by layering the translation mixture (60 μl) on a 4-ml step gradient of 1-ml intervals of 77% (wt/vol), 30%, 20%, and 10% sucrose containing 20 mM Hepes (adjusted to pH 7.5 with NaOH)/2 mM dithiothreitol for fractionation by centrifugation (50,000 rpm, 2 hr at 4°C; Sorvall model TsT 60.4 rotor). SDS/PAGE located the membrane-bound L protein predominantly at the 77%/30% sucrose interface. Sucrose gradient-purified L protein (4 μl) was diluted with NET-gel buffer (100 μl) containing various dilutions of either antibody or peptide for inhibition assays. Mixtures were incubated in HBcAg-coated wells (as in the paragraph *Isolation of Phage that Bind to HBcAg*) for 2 1/2 hr at 4°C and washed five times with NET-gel buffer with 10-min intervals. Wells were placed in scintillation vials containing Ecoscint A (5 ml; National Diagnostics) for quantitation of radioactivity.

RESULTS AND DISCUSSION

Screening a Phage Library for Peptides Binding to HBcAg. Hexapeptide ligands that bind HBcAg particles have been isolated from a random fusion phage display library made by Scott and Smith (1). Three rounds of affinity selection against HBcAg resulted in the enrichment of specific fusion phages. In two experiments, A and B, the phage library was screened against truncated HBcAg (aa 3–148) and in a third experiment, C, against BSA. In experiment A the washing buffer used during affinity purification contained 0.15 M NaCl, whereas for experiments B and C this concentration was increased to 0.5 M. In the case of experiment B, 10^{-3}% of phage bound to the membrane in the first round of panning, and this binding increased to 1% in the third panning. No increases were observed in experiments A and C.

The peptides carried by phage cloned from various eluent pools are shown in Table 1. Panning the phage library against BSA gave apparently random peptide sequences (experiment C). This result is as expected because the library had been incubated with a buffer containing BSA, before panning, to eliminate phage that bind BSA. No recognizable consensus sequence was observed in initial affinity selection of the library against HBcAg (experiment A), but a strong conservation of sequence between selected phage clones became apparent when the selection was made more stringent by washing at higher salt concentration. Of 46 independent clones, 36 were very similar (experiment B) with the sequence LLGRMK (clone B1) predominating, followed by the somewhat similar sequence YLLRFR (clone B2). Conservative variations of B1 were also observed with the methionine at position 5 substituted by leucine or phenylalanine and the lysine at position 6 substituted by arginine.

Specificity of Selected Phage for HBcAg. To show that the solid-phase selection system for phage that bind HBcAg

Table 1. Peptides binding to HBcAg or BSA selected from the phage library

Exp. A		Exp. B		Exp. C	
1	MFLRAG	1	LLGRMK (15)	1	ANGCCD
2	LHADVW	2	YLLRFR (11)	2	HAILNI
3	QLYMKG	3	LLGRLK (6)	3	ESQSVL
4	WAMSEV	4	LLGRFK (2)	4	RVADIV
5	EPGLDR	5	LLGRFR (2)	5	SKGTVA
6	SFNILR	6	LLGRLR	6	DVSIGV
7	PVQASP	7	LLGRMR	7	RYLVLE
8	DSASHS	8	SLWKWK	8	NINSPV
9	SPCRGL	9	WTFLRG	9	IWLSNG
10	GWWPHR	10	Unrelated (6)	10	WPRGAG

For Exps. A and B, phage were selected with truncated HBcAg, and in Exp. C phage were selected with BSA. The number of clones encoding the same peptide is shown in parentheses.

reflected a reaction with native HBcAg particles rather than a dissociated or denatured form, cloned phage were incubated in solution with various concentrations of freshly prepared HBcAg and then placed in polystyrene wells coated with HBcAg particles. Fig. 1*A* shows that preincubation with increased concentrations of HBcAg progressively reduced the availability of phage for binding to HBcAg on the solid phase, proving that the phage did indeed recognize intact HBcAg particles. Relative dissociation constants (K_d^{Rel}) between the various fusion phage and HBcAg in solution (Table 2) were calculated by curve-fitting the data in Fig. 1 to a hyperbolic function (Sigma Plot, Jandel, Erkrath, Germany). This method follows that used for measurement of affinity constants in solution between antigen–antibody complexes (25) and will be described in detail elsewhere. The equilibrium dissociation constants obtained are *relative* numbers because both HBcAg and the phage are multivalent. Table 2 shows the order of binding of the hexapeptide ligands to HBcAg to be LLGRMK > LLGRFK > LLGRLK > YLLRFR.

Fig. 1*B* shows that phage B1 bound to full-length HBcAg (aa 3–183) as efficiently as to truncated HBcAg but did not bind to heat-denatured, truncated HBcAg. The selected fusion phage may thus recognize a conformational feature, perhaps a cleft, within the native, particulate form of HBcAg rather than a linear contiguous amino acid sequence. Further, the detailed surface of the particles of the truncated and full-length HBcAg molecules (26) must be very similar because the ligand binds the two structures with similar affinities.

A Peptide Ligand for HBcAg. Can the hexapeptide LLGRMK bind HBcAg in the absence of the gpIII protein context of the filamentous phage? Fig. 1*C* shows that the

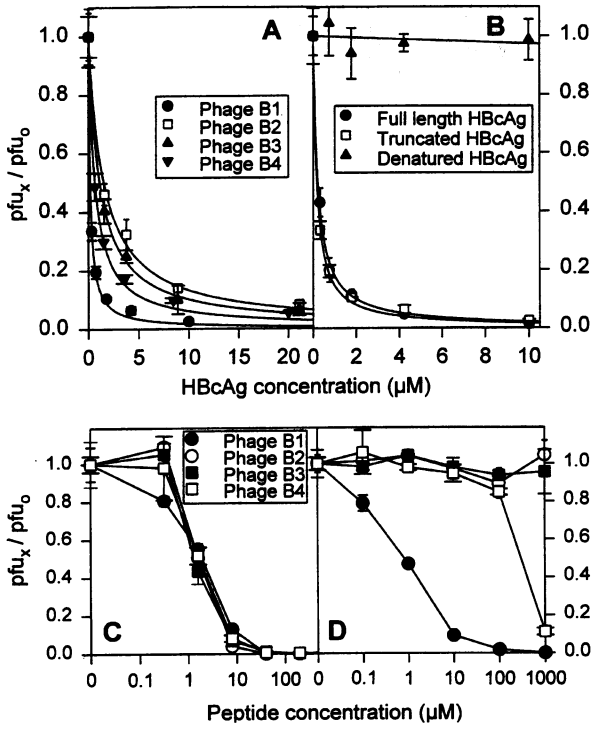

FIG. 1. Inhibition of fusion phage binding to HBcAg. Phage were incubated in HBcAg-coated wells, and bound phage were determined by titering eluted pfu. pfu$_x$, Number of phage eluted in the presence of ×M inhibitor; pfu$_o$, number of phage eluted in the absence of inhibitor. (*A*) Phage B1–4 were incubated with various concentrations of HBcAg. (*B*) Phage B1 was incubated with various concentrations of either full-length HBcAg, native truncated HBcAg, or heat-denatured truncated HBcAg. (*C*) Phage B1–4 were incubated with various concentrations of peptide ALLGRMKG. (*D*) Phage B1 were incubated with various concentrations of peptide ALLGRMKG (●), ALLTRILG (○), GRMKG (■), or LDPAFR (□).

Table 2. Relative dissociation constants of the phage–HBcAg complexes

Phage	Sequence	Relative dissociation constants,* μM	
		Truncated HBcAg	Full-length HBcAg
B1	LLGRMK	0.17 ± 0.01	0.22 ± 0.01
B2	YLLRFR	1.53 ± 0.10	ND
B3	LLGRLK	1.13 ± 0.05	ND
B4	LLGRFK	0.64 ± 0.03	ND

ND, not determined.
*Calculated from the data of Fig. 1.

octapeptide ALLGRMKG inhibits the binding of fusion phage B1, B2, B3, and B4 to HBcAg-coated polystyrene wells with 50% inhibition at a peptide concentration of 1 μM, a concentration ≈6-fold higher than the K_d^{Rel} for the fusion phage–HBcAg complexes. This difference may be attributable to the monovalency of the peptide inhibitor compared with the multivalency of the HBcAg inhibitor. The two flanking amino acids of the gpIII protein were added to the selected hexapeptide sequence because this can increase the binding affinity of phage-selected peptides (6).

Because phage bearing the sequence LLGRMK bound to native HBcAg particles in solution, the sequence could represent one loop of the binding site of an antibody to HBcAg, but more interestingly it may resemble a region of the HBsAg polypeptide that contacts the nucleocapsid in the intact virion. Two 3-of-6 matches of the sequence were identified, one at positions 21–27 of S (LLTRIL) and one at positions 63–65 of the pre-S1 region (LLG). However, neither peptide ALLTRILG (corresponding to aa 21–27 of S) nor GRMKG (the carboxyl segment of the selected peptide) inhibited binding, but the peptide LDPAFR (corresponding to aa 19–24 of the pre-S1 region of L and the epitope for monoclonal antibody 18/7) exhibited 50% inhibition at a concentration of ≈350 μM (Fig. 1*D*). This result shows that neither the hydrophobic amino nor the hydrophilic carboxyl half of peptide ALLGRMKG alone is sufficient for binding to HBcAg, but both regions play a role in complex formation. The hexapeptide LLGRMK may, therefore, be a mimic of internal regions of HBsAg that contact HBcAg and which include amino acids of HBsAg which are near to each other in the folded protein but separated in primary sequence. All the sequences selected for HBcAg binding exhibited a net charge of +2, suggesting that a basic region of HBsAg may contact an acidic external area of the nucleocapsid.

HBsAg L Polypeptide Forms a Complex with HBcAg. The hypothesis that the sequences selected for binding to HBcAg represent a mimotope of the HBV envelope proteins was tested in an assay designed to resemble a stage of morphogenesis involving the association of HBcAg with HBsAg. HBcAg and the 22-nm particles of HBsAg interact poorly, if at all, as judged by sedimentation analysis (K.M., and D. Hubsch, unpublished data) and so our assay involved the synthesis of either L or S HBsAg by *in vitro* translation and the association of these nascent products with HBcAg. L and S forms of HBsAg were translated from transcripts from a DNA template in a rabbit reticulocyte lysate, supplemented with [^{35}S]methionine, in the presence or absence of microsomal vesicles. In the presence of microsomes, translation of S HBsAg mRNA produced bands on SDS/PAGE (Fig. 2*A*, lane 2) of 24 kDa and 27 kDa, corresponding to the unglycosylated and glycosylated forms of the S form, and translation of L HBsAg mRNA gave a predominant band at 39 kDa, corresponding to the unglycosylated protein, and a fainter band at 42 kDa, corresponding to the glycosylated form (Fig. 2*A*, lane 4). The glycosylated polypeptides were not obtained in the absence of microsomes. The glycosylated L and S sequences are the same size as those found in native HBV, indicating similar topology,

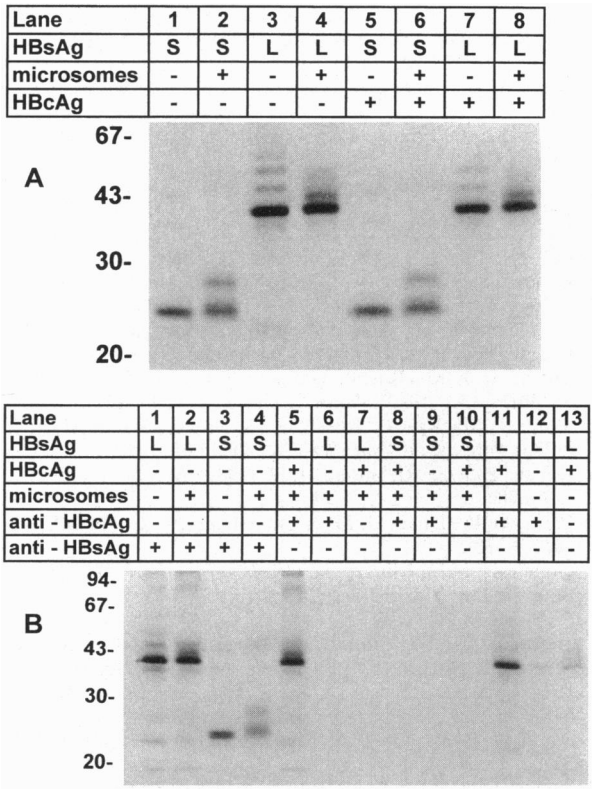

FIG. 2. Translation and immunoprecipitation of S and L HBsAgs. *In vitro*-synthesized RNAs for S and L HBsAgs were translated in reticulocyte lysates, containing [^{35}S]methionine, in the presence (+) or absence (−) of microsomes and 0.1 μg of HBcAg. ^{35}S-labeled products were examined by SDS/PAGE and autoradiography. Numbers at left are molecular mass standards (kDa). (*A*) Lanes 1, 2, 5, and 6 show the translation products with HBsAg S mRNA, and lanes 3, 4, 7, and 8 show those with HBsAg L mRNA. (*B*) Translated products immunoprecipitated with either polyclonal anti-HBsAg (lanes 1–4) or polyclonal anti-HBcAg (lanes 5–13).

with respect to the membrane, to the natural forms, in accord with a recent report (27).

Translation reactions were carried out in the presence or absence of HBcAg, and the products were immunoprecipitated with anti-HBcAg polyclonal rabbit serum. L polypeptide was precipitated with anti-HBcAg only in the presence of HBcAg, demonstrating complex formation between the newly synthesized membrane-bound L polypeptide and HBcAg (Fig. 2*B*, lane 5) and that this step could be separated from other stages of viral assembly. The L polypeptide was still able to form a complex with HBcAg in the absence of microsomal vesicles (Fig. 2*B*, lane 11), suggesting that the pre-S regions of the L polypeptide may constitute an independently folded domain, not requiring insertion of the S region into a membrane for its ability to bind to HBcAg. In contrast, the S form was not precipitated with anti-HBcAg in the presence of HBcAg (Fig. 2*B*, lane 8). Included as a positive control, both L and S polypeptides were precipitated with anti-HBsAg polyclonal rabbit sera (Fig. 2*B*, lanes 1–4).

Because HBcAg bound L HBsAg, but did not bind S HBsAg, complex formation must involve the pre-S domain. Fig. 3 illustrates diagramatically the S-domain topology in the membrane (27–30) and the proposed docking of the nucleocapsid with the pre-S1 domain. The failure of S HBsAg to associate directly with HBcAg may explain the abundance of the 22-nm HBsAg particles, which contain predominantly the S form, in the sera of infected patients and may indicate different secretion pathways for the L and S HBsAgs. The requirement of both L and S HBsAg for virus formation in tissue culture (7, 8) points to interactions between L HBsAg and S HBsAg in a separate step of viral assembly.

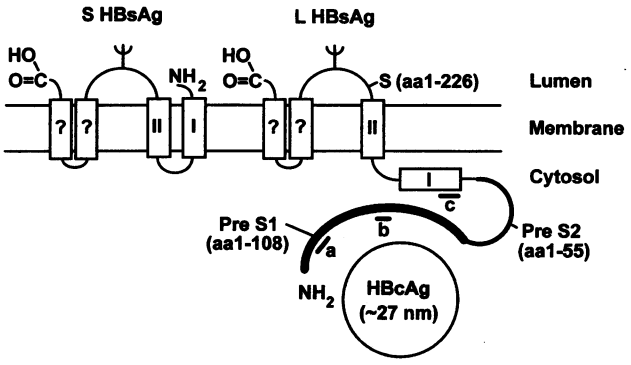

FIG. 3. Domain structure of L and S HBsAgs and association with HBcAg. The diagram (adapted in part, with permission, from ref. 27) represents the positions of the pre-S1, pre-S2, and S regions of L HBsAg, topogenic signal sequences I and II, glycosylation site (aa 146; fork), and the proposed docking of HBcAg with the pre-S1 domain. Regions of interest are as follows: a, LDPAFR (aa 19–24); b, LLG (aa 63–65) in pre-S1; and c, LLTRIL (aa 21–26) in the S domain.

Inhibitors of the Association of HBsAg with HBcAg. The assay system developed for inhibitors of the interaction between HBsAg and HBcAg involved incubating purified membrane-inserted ^{35}S-labeled L polypeptide in HBcAg-coated wells and quantitating bound protein by scintillation counting. The specificity of this interaction was established through its inhibition by various antibodies. Fig. 4*A* shows that anti-HBcAg polyclonal serum inhibited the association of L HBsAg with HBcAg at a two-logarithmic higher dilution than preimmunized polyclonal serum from the same rabbit. Polyclonal serum raised against denatured S HBsAg inhibited the association reaction at a 1.5-logarithm-higher dilution than polyclonal serum against the native S HBsAg. The principal target for polyclonal antibodies to the native, particulate S HBsAg resides between aa 110 and aa 150 (31–33). That antisera against denatured S HBsAg effectively inhibited the interaction between HBcAg and L HBsAg, whereas antibodies to native (S) HBsAg do not, probably reflects the presence of antibodies specific for cytoplasmically disposed epitopes of the membrane-bound S HBsAg that are normally hidden in the native form. Interestingly, the most effective inhibitor of the interaction between the two antigens was a monoclonal anti-

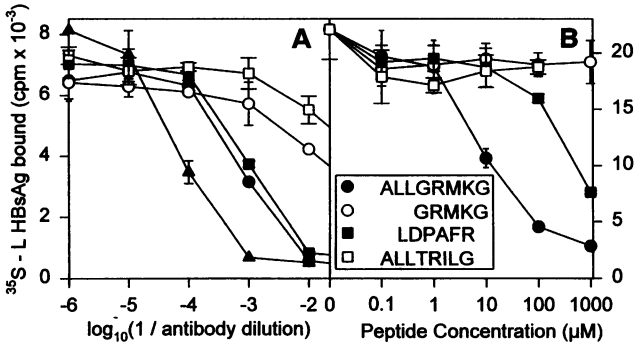

FIG. 4. Inhibition of L HBsAg binding to HBcAg by antibodies and peptides. ^{35}S-labeled L HBsAg, in microsomes, was incubated in HBcAg-coated wells, and bound ^{35}S-labeled L HBsAg was quantitated by scintillation counting. Points represent the average of three experiments, and error bars represent the SEMs. (*A*) Reactions were done in the presence of various dilutions of either neutral polyclonal rabbit serum (□), polyclonal serum raised against HBcAg (●), native HBsAg (○), denatured HBsAg (■), or monoclonal antibody 18/7 (1 mg/ml) (▲). (*B*) Incubations were done with various concentrations of peptides.

body (18/7) specific for a region close to the amino terminus of the pre-S1 domain, aa 20–23 (4, 34), not previously thought necessary for virus formation (35). However, large molecules, such as immunoglobulins, can cause steric hindrance over a considerable distance in any compound to which they bind.

The synthetic peptide ALLGRMKG, identified as a ligand to HBcAg via the fusion phage library, inhibited the reaction between the L HBsAg and HBcAg with 50% inhibition observed at a peptide concentration of 10 μM (Fig. 4*B*). The peptides GRMKG and ALLTRILG (the latter including aa 21–27 of the S region) exhibited no inhibitory properties, in accord with their inability to inhibit binding of fusion phage to HBcAg. However, LDPAFR, which includes the epitope (residues 20–23) recognized by a monoclonal antibody (18/7) to the pre-S1 domain, did inhibit, but with a half-maximal effect at $\approx$360 μM.

The results provide evidence that sequences selected from the fusion phage library for binding to HBcAg do, indeed, mimic cytoplasmic regions of L HBsAg. Taken together with the observation of inhibition by monoclonal antibody 18/7, or a peptide containing its epitope, these results show that at least part of the contact region for HBcAg lies within the pre-S1 domain (Fig. 3). The inhibition may be direct, with the peptides binding residues of HBcAg normally involved in HBsAg binding, or the peptides may bind to an alternative site and alter the conformation of the L HBsAg-binding domain in an allosteric manner. It should now be possible to map the binding site of the selected peptide on HBcAg, by chemical cross-linking, to identify specific amino acids of HBcAg that may be involved in recognition of the L HBsAg. An equivalent series of experiments with L HBsAg preparations may identify a corresponding mimotope of the binding domains of HBcAg. This approach should be generally applicable, not only to viral assembly, but to other complex biological assemblies such as ribosomes, spliceosomes, nucleosomes, proteasomes, and transcription complexes.

In addition to contributing to our understanding of HBV morphogenesis, the selected peptide ALLGRMKG may represent a lead antiviral agent targeted at the inhibition of viral assembly; we are attempting to evaluate this in transformed hepatoma cells that produce the virus. Such compounds could be useful in chronic infections. The search for effective therapeutic agents against diseases associated with HBV has become more urgent with the recent emergence of escape mutants of HBV (36) that are not neutralized by vaccine-induced antibodies. These approaches are encouraged by the recent demonstration that peptides taken from viral components can inhibit influenza (37), sindbis, and vesicular stomatitis (38) virus formation.

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