Optimal Conditions for Elution of Hepatitis B Antigen After Adsorption onto Colloidal Silica

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Hepatitis B surface antigen (HB_sAg) adsorbed from sera onto colloidal silica could be completely eluted through the use of 0.25% sodium deoxycholate in 0.01 M borax, pH 9.3, at 56°C. The HB_sAg recovered in the eluate represented 100% of that present in the original serum, and it was contaminated by only trace amounts of serum proteins (in decreasing amounts: β -lipoprotein, immunoglobulin G, albumin). This preliminary step greatly facilitates purification of large amounts of HB_sAg and provides small volumes of highly concentrated material for subsequent purification by density gradient centrifugation.

The possibility exists of immunizing humans with hepatitis B surface antigen (HB_sAg), since it has clearly been established that the chimpanzee can be immunized using this fraction of the virus particle (4). For this purpose it is necessary to purify large amounts of HB_sAg. The most commonly used methods consist of isopycnic banding in a CsCl density gradient, followed by rate zonal centrifugation on a sucrose density gradient.

It is cumbersome to submit large volumes of plasma or serum containing HB_sAg to ultracentrifugation as the first step of purification. Some workers have precipitated the HB_sAg with polyethylene glycol 6000 (1). To precipitate the HB_sAg entirely, it is necessary to use concentrations of polyethylene glycol 6000 up to 10 or 15% at neutral pH, and under these conditions, many serum proteins also precipitate.

Duimel et al. (2) and Siebke et al. (7) have shown that all the HB_sAg contained in a serum (22-nm spherical, tubular, and 42- to 45-nm Dane particles) could be adsorbed on colloidal silica and then eluted either with a low ionic strength buffer (0.01 M borax, pH 9.3) (2) or with a saturated solution of sodium chloride [in 1 M tris(hydroxymethyl)aminomethane-hydrochloride buffer, pH 9] (7). We have obtained the same results as concerns adsorption; however, we could not recover in the eluate all of the HB_sAg initially present in the serum and entirely adsorbed onto the colloidal silica. Elution with low ionic strength buffer gave better results than did elution with saturated sodium chloride solutions in tris(hydroxymethyl)aminomethane-hydrochloride buffer, but at best only a portion of the adsorbed HB_sAg was recovered.

We have studied conditions for elution of

HB_sAg adsorbed onto colloidal silica and have been able to elute all of the adsorbed HB_sAg using a low ionic strength buffer containing sodium deoxycholate. The eluate contained 100% of the antigen initially present in the serum and minimal amounts of contaminating serum proteins.

MATERIALS AND METHODS

HB_sAg was identified and quantitated by means of electrosyneresis. Adsorption of HB_sAg was performed either on whole serum or on plasma; when plasma was used it was first coagulated. Twenty milligrams of colloidal silica (Aerosil 380, Serlabo, Paris), washed twice in distilled water, was added to 1 ml of serum. The mixture was incubated at 37°C for 3 h, with mild agitation, and then centrifuged for 10 min at $4,000 \times g$. The pellet was then washed three or four times with 2 to 3 volumes of saline until the $E_{280}^{1.5\text{cm}}$ of the washings was less than 0.04. During the washings, the HB_sAg remained firmly bound to the colloidal silica. Elution was performed by adding one-half volume of the test solution twice, and the two eluates were then pooled. The amount of protein extracted was estimated from the $E_{280}^{1.5\text{cm}}$ or by the biuret method. The nature of serum proteins present in the eluates together with the HB_sAg was determined by immunoelectrophoresis and immunodiffusion using a potent antiserum to normal human serum (Hyland) and a panel of monospecific antisera produced by Behring. Those proteins present in sufficient amounts quantitated by radial immunodiffusion (using proteiplaques, Institut Pasteur Production, Paris). Under the test conditions, all of the HB_sAg was usually adsorbed; the HB_sAg in sera with titers as high as 1:160 by electrosyneresis was entirely adsorbed. However, among 100 different sera treated by this method, two contained HB_sAg that did not adsorb, or only partially adsorbed. We have no satisfactory explanation for this observation.

RESULTS

Elution from colloidal silica with 0.01 M borax, pH 9.3, at 37°C (2) gave a maximum of only 60% recovery of the HBsAg initially present in the serum, indicating that approximately 40% of the HBsAg adsorbed remained firmly bound to the colloidal silica and thus escaped elution with this buffer. Often such a yield could be obtained only after three to five successive treatments of the colloidal silica pellet with the elution buffer. Increasing the ionic strength of the borax up to 0.5 M at constant pH (pH 9.3) resulted in a decrease of the yield. Chaotropic ions (3, 6) at concentrations near saturation in distilled water (3 M NaCl, 5 M MgCl₂, 3 M NaI) only released small amounts of adsorbed HBsAg. Acid buffers (0.1 M glycine-hydrochloride, pH 2.5, 0.1 M acetic acid) were not more effective. Addition of 10% ether to the borax did not improve the yields of HBsAg in the eluates.

The temperature at which elution is performed is an important factor to be considered. We compared elution with borax at 37, 56, and 65°C. Results are shown in Table 1. The amount of HBsAg eluted at 56°C and higher was greater than that eluted at 37°C. In addition, with an increase in temperature, we observed a decrease in elution of some serum

proteins, particularly albumin, which often represented the major contaminating protein eluted along with HBsAg.

Increase of temperature, however, never led to recovery of all of the adsorbed HBsAg.

Numerous trials, consisting of the addition of nonionic detergents to the borax, led us to select sodium deoxycholate. As shown in Table 2, addition of 0.25 or 0.5% sodium deoxycholate to the borax resulted in elution of all the adsorbed HBsAg; in the first elution almost all of the adsorbed HBsAg was retrieved. Along with this increase in HBsAg, we observed an increase in the amount of β -lipoprotein eluted, the concentration of which seemed to be proportional to the concentration of deoxycholate used. Considering these results, we propose removal of HBsAg by means of two successive elutions at 56°C with borax containing 0.25% deoxycholate. This temperature was selected because we noticed that elution of serum contaminants was lower than at 37°C, even in the presence of deoxycholate.

DISCUSSION

Complete elution of HBsAg adsorbed onto colloidal silica was accomplished by treating the adsorbant with borax containing 0.25% deoxycholate. The effect of deoxycholate is prob-

TABLE 1. *Effect of temperature on the elution of HBsAg adsorbed onto colloidal silica^a*

Elution temp ^a (°C)	Titer of HBsAg in the eluate (E.S.) ^b	$E_{280}^{1.5\text{cm}}$	Proteins eluted				
			Dosable (mg/ml)		Not dosable		
			Albumin	IgG	IgM ^c	IgA	C3
37	1/4	3.10	3.1	0.33	+	+	+
56	1/8	2.95	1.2	0.40	+	—	—
65	1/8	2.90	0.8	0.43	+	—	—

^a HBsAg was eluted with 0.5 volume borax applied twice; both eluates were pooled.

^b E.S., Electrosyneresis.

^c In some experiments, IgM, IgA, and C3 were not detectable; it was also sometimes possible to detect trace amounts of α -lipoprotein and α_2 -macroglobulin.

TABLE 2. *Effect of the concentration of sodium deoxycholate on the elution of HBsAg adsorbed onto colloidal silica*

Concn of sodium deoxycho- late (%)	Titer of eluted HBsAg (E.S.) at ^a		Total proteins (biuret) (mg/ ml)		Eluted proteins (mg/ml) ^b							
					Albumin at:		β -Lipoprotein at:		IgG at:		IgM at:	
	37°C	56°C	37°C	56°C								
					37°C	56°C	37°C	56°C	37°C	56°C	37°C	56°C
0.25	1/18	1/27	3.1	2.6	0.10	0.075	1.05	0.30	0.25	0.22	<0.11	0
0.50	1/27	1/27	3.8	3.5	0.075	0.075	1.50	0.30	0.24	0.38	<0.11	0
1.00	1/18	1/27	5.2	3.2	0.09	0.09	0.30	0.95	0.56	0.31	<0.11	0

^a E.S., Electrosyneresis

^b α -Lipoprotein and α_2 -macroglobulin were not detected when sodium deoxycholate was used; trace amounts of immunoglobulin A, C3, and C4 were detected with some sera.

ably upon the hydrophobic forces that maintained the HBsAg adsorption. The relative specificity of deoxycholate is similar to its specificity in removing certain enzymes during hydrophobic chromatography (8). By the method described it is possible to concentrate into a small volume all of the HBsAg in a serum, contaminated by only minimal amounts of serum proteins. This greatly facilitates further purification steps in processing large amounts of HBsAg. β -Lipoprotein was the major contaminating protein, but it was present at less than one-eighth of the initial serum concentration. Immunoglobulin G (IgG) was also always detected. This presence of IgG was not related solely to the presence of circulating immune complexes (5), since IgG was also always detected, in comparable amounts, after adsorption and elution of normal human serum on colloidal silica. On the other hand, the variable trace amounts of IgM, IgA and C3, depending upon the sera used, might relate to antibody and complement forming immune complexes with HBsAg. β -Lipoprotein, which was undetectable when elution was performed without deoxycholate, appeared when deoxycholate was used for elution.

Electron microscopy showed that treatment with sodium deoxycholate does not modify the

morphology of HBsAg 22-nm particles.

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