Purification of the Envelope Glycoproteins of Western Equine Encephalitis Virus by Glass Wool Column Chromatography

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Glass wool column chromatography was used for separation of the two glycoproteins of western equine encephalitis virus. Cross-contamination of each protein separated was confirmed to be negligible by sodium dodecyl sulfate-polyacrylamide gel electrophoresis.

Western equine encephalitis (WEE) virus is a member of the toga-alphavirus group, in which the nucleocapsid is surrounded by a lipid bilayer membrane. The alphavirus glycoproteins E1 and E2 are anchored in the lipid bilayer by short hydrophobic segments, and one or both extend completely through the membrane (4, 6). It has been suggested that the two envelope proteins form a complex in the viral membrane and remain associated with each other when solubilized with nonionic detergents, which are known to solubilize membrane proteins without disruption of native structure or loss of biological activities (5, 9). So far several procedures have been tried to isolate and separate the two glycoproteins of alphaviruses without loss of biological activities or antigenicities (2, 3, 7, 11). Recently, Bell et al. (1) reported the separation of the two glycoproteins of Sindbis virus on the basis of their differential binding to glass wool in the presence of the nonionic detergent Triton X-100.

We are currently investigating the biological function of alphavirus glycoproteins, and we tried to adapt the method described by Bell et al. to the separation of the E1 and E2 proteins of WEE virus, the virus most closely related immunologically to Sindbis virus. We report here that glass wool column chromatography is very useful for the separation of the two glycoproteins of WEE virus and may be applicable to other togaviruses.

MATERIALS AND METHODS

Propagation and purification of WEE virus. WEE virus was grown in chicken embryo fibroblast cells as described previously (10). Infected culture fluids were concentrated and purified by differential centrifugation and sucrose density centrifugation (8). A small amount of radioactive virus labeled with [³H]leucine (10) was used as a marker.

Purification of virus proteins. One-tenth volume of 10% Nonidet P-40 (Shell Chemical Co.) was added to the purified virus sample, previously pelleted by differential centrifugation to remove sucrose, and suspended in TNE buffer [10 mM tris(hydroxymethyl)aminomethane hydrochloride–0.14 M NaCl–1 mM ethylenediaminetetraacetic acid, pH 8.0] and allowed to stand at room temperature for 20 min. Whole-virus lysate containing envelope glycoproteins E1 and E2 and nucleocapsid (core) protein was centrifuged at $175,000 \times g$ for 30 min to pellet the core protein. The supernatant containing E1 and E2 proteins was dialyzed against equilibration buffer (0.5 M NaCl–0.3 M KH_2PO_4–Na_2HPO_4–1 mM ethylenediaminetetraacetic acid–0.2% Nonidet P-40, pH 6.5) before application to a glass wool column.

Glass wool column and chromatography. Pyrex glass wool (Corning Glass Works) was sheared to short fibers at high speed in a Waring blender or ground to very small fibers (but not to powder) by a glass mortar (1). Forty grams of sheared glass wool was packed into a 1.5- by 33-cm column. Before use, the column was washed with 1 liter of 1% sodium dodecyl sulfate and equilibrated with equilibration buffer. The column could be used repeatedly by washing extensively with equilibration buffer before use.

The supernatant of the $175,000 \times g$ centrifugation containing E1 and E2 proteins was applied to the column, washed with equilibration buffer to flow through the E1 protein, and then eluted with elution buffer [1.0 M NaCl–0.1 M tris(hydroxymethyl)aminomethane hydrochloride–1 mM ethylenediaminetetraacetic acid–0.2% Nonidet P-40, pH 9.0] to elute the E2 protein; any material remaining bound to the column was recovered by the chasing buffer [0.5 M NaCl–0.5% sodium dodecyl sulfate–0.05 M tris(hydroxymethyl)aminomethane hydrochloride, pH 9.0]. One-milliliter fractions were collected throughout the entire procedure.

HA test and polyacrylamide gel electrophoresis. The hemagglutination (HA) test was done as described elsewhere (7), using 1-day-old chicken erythrocytes instead of goose erythrocytes. Sodium dodecyl sulfate-polyacrylamide (7.5%) gel (Bio-Rad) electrophoresis was performed by the discontinuous buffer system of Laemmli, as described previously (7).

RESULTS

Preliminary experiment using the procedures for separation of Sindbis virus gly-

coproteins. To determine the best conditions for separating each glycoprotein of WEE virus, the equilibration and elution buffers were tested. When we used the equilibration buffer for the glycoproteins of Sindbis virus (0.3 M NaCl, pH 5.5) (1), the E1 and E2 glycoproteins of WEE virus were all retained in glass wool. Therefore, we examined several buffers of different ionic strengths and pH for a suitable equilibration buffer which would make the E1 protein flow through but the E2 protein be retained in glass wool; this resulted in the equilibration buffer (0.5 M NaCl + 0.3 M phosphate, pH 6.5) for the glycoproteins of WEE virus as described in Materials and Methods. We also tested a suitable buffer for elution of the E2 protein of WEE virus. For this purpose, tris(hydroxymethyl)-aminomethane hydrochloride buffers containing 0.5 to 1.0 M linear NaCl gradients at pH values of 8.0, 8.5, and 9.0, respectively, were tested. As a result, we chose higher ionic strength and pH conditions for the elution buffer (1 M NaCl, pH 9.0) than those (0.8 M NaCl, pH 8.0) used for Sindbis virus (see Materials and Methods).

Purification of the two glycoproteins of WEE virus. Figure 1 shows typical elution patterns of the E1 and E2 glycoproteins of WEE virus by glass wool column chromatography.

There are two major peaks and one minor peak with radioactivity. An analysis of pools of the fractions of the three peaks by polyacrylamide gel electrophoresis is shown in Fig. 2. These two figures show that the E1 protein did not bind to glass wool, but rather flowed through the column and was found in the equilibration buffer wash; the E2 protein was recovered in the elution buffer containing high salt and Nonidet P-40 and, to a lesser extent, in the chasing buffer with sodium dodecyl sulfate. Cross-contamination was negligible (Fig. 2). HA activity was associated only with the E1 protein, as described previously (7).

The recovery rate of both glycoproteins was calculated as 81.9% on the basis of radioactivity (Table 1). The recovery of the E1 protein was always higher than that of the E2 protein. When we rechromatographed the E1 protein, more than 90% was recovered (data not shown). The E2 protein was not released completely from the glass wool even when we used high salt and pH buffer as the elution buffer.

DISCUSSION

In our laboratory, the two envelope glycoproteins E1 and E2 of WEE virus have been sepa-
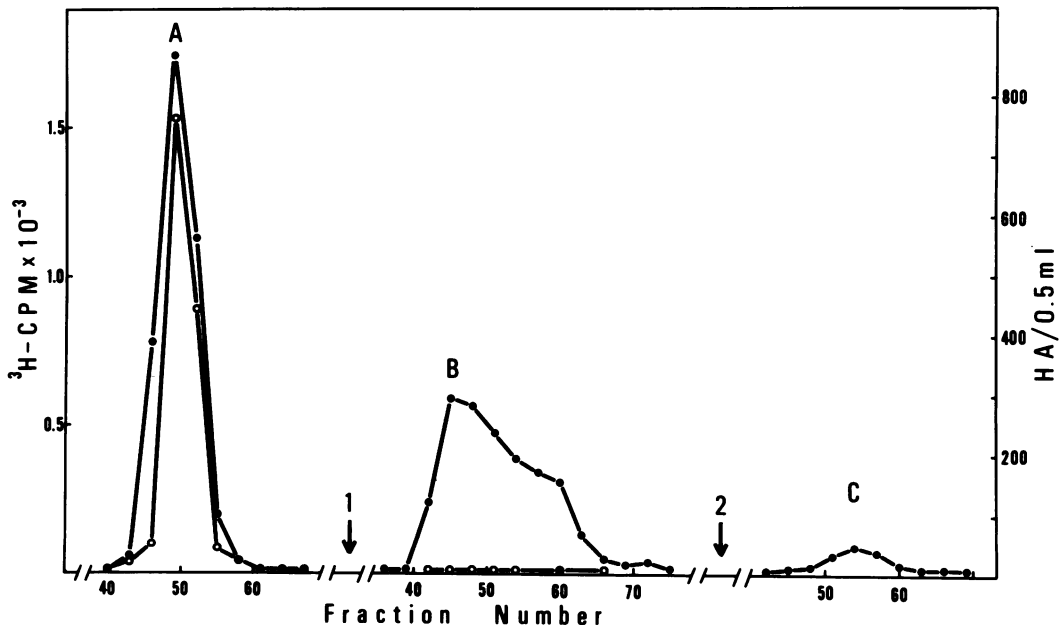

FIG. 1. Chromatography of the E1 and E2 glycoproteins of WEE virus on a glass wool column (1.5 by 33 cm). The 175,000 × g supernatant containing the E1 and E2 protein, 6.8 mg/2.8 ml (54,600 cpm), was applied to the column, which was then washed with equilibration buffer. Buffer changes are indicated by arrows: 1, elution buffer was begun; 2, chasing buffer was begun. The column was operated at a flow rate of approximately 1 ml/10 min, and 1-ml fractions were collected. Symbols: ●, counts per minute/0.5 ml; ○, HA units per 0.5 ml.
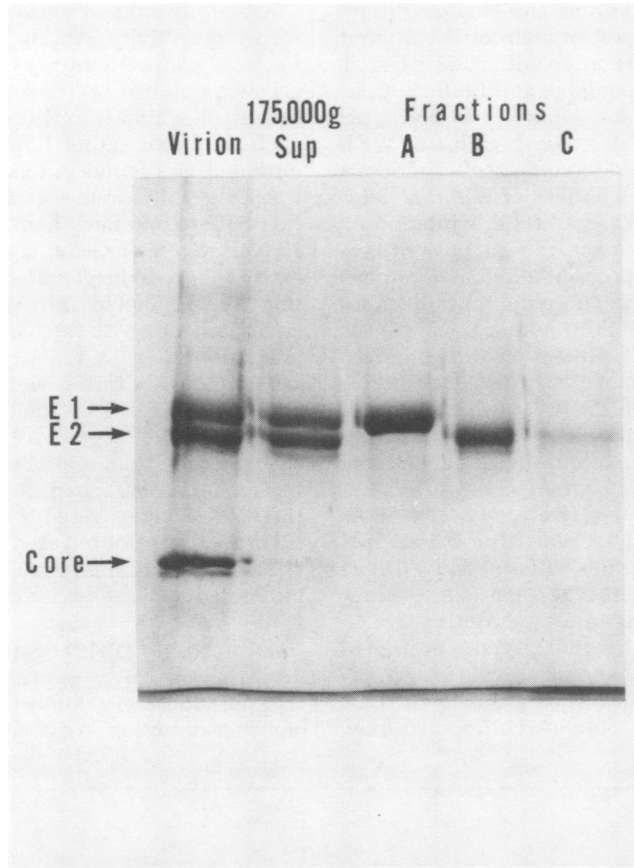
FIG. 2. *Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of pooled fractions from glass wool chromatography shown in Fig. 1. Applied samples were dialyzed against TNE buffer before electrophoresis, denatured with 1% sodium dodecyl sulfate–5 mM mercaptoethanol, and heated at 70°C for 30 min; they were then electrophoresed as described previously (7). Fractions B and C were collected, put into cellulose tubing (Visking Co.), and embedded in Ficoll-400 (Pharmacia Fine Chemicals) for concentration before application to the gel. After electrophoresis the gel was stained with Coomassie brilliant blue.*

rated by isoelectric focusing on urea gradients in the presence of Triton X-100 (7). Also, we have accomplished separation of the solubilized gly-coproteins by using different pH and ionic strength conditions, according to Burke and Keegstra (2). The former procedure requires more time, and the latter method is simple and useful but cross-contamination of the E1 and E2 proteins must be taken into consideration.

We examined glass wool column chromatog-raphy, developed by Bell et al. (1) for separation of Sindbis glycoproteins, and found that this method is simple and extremely useful for large-scale purification of the two glycoproteins of WEE virus without loss of biological activity and antigenicity. Therefore, this method may be applicable to other togaviruses with slight mod-ification of the equilibration and elution buffer systems.

Our results demonstrated that the E1 protein

was always recovered as a single and sharp peak but the E2 protein was eluted in various amounts, depending on the conditions used. This leads us to suspect that the E2 proteins of WEE and Sindbis (1) viruses may have heterogeneous binding activity to glass wool, although the E2 proteins of both viruses are not heterogeneous when analyzed by sodium dodecyl sulfate-poly-acrylamide gel electrophoresis.

The recovery of HA activity was only 7% of the applied sample. This may result in loss of lipids associating with the E1 protein (hemag-glutinin) or conformational changes of the pro-tein caused by flowing through glass wool rather than in inactivation of the activity, because HA activity of the E1 protein eluted from the glass wool column was recovered by the addition of lipids obtained from virions (manuscript in prep-aration).

It was noted that when the glass wool column

TABLE 1. *Recovery of the envelope glycoproteins of WEE virus by glass wool column chromatography*

Sample[a]	Recovery	
	Radioactivity (cpm)	HA activity (HAU)[b]
Applied[c]	54,600 (100%)	143.4×10^3 (100%)
Fraction A[d]	23,958 (43.9%)	10.1×10^3 (7.1%)
Fractions B + C[e]	20,742 (38.0%)	Not detectable
Total (A + B + C)	44,700 (81.9%)	10.1×10^3 (7.1%)

[a] See the legend to Fig. 1.

[b] HAU, Hemagglutinating units.

[c] Applied total radioactivity and HA activity.

[d] Recovered counts per minute and hemagglutinating units from fractions 43 to 61 in Fig. 1.

[e] Recovered counts per minute from B, fractions 40 to 70, and C, fractions 48 to 62, in Fig. 1.

was used repeatedly, the flow rate of the elution buffer became slow and very little E2 protein was eluted because of shortened glass fibers. This agrees with the fact that the more finely the glass wool is sheared, the more strongly the E2 protein binds to the glass (1).

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