The Interaction of Encephalomyocarditis Virus with its Erythrocyte Receptor on Affinity Chromatography Columns

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SUMMARY

Glycophorin, the major sialoglycoprotein in the human erythrocyte surface membrane, can serve as a red cell receptor for both wheat-germ agglutinin (WGA) and encephalomyocarditis (EMC) virus since glycophorin bound to WGA–Sepharose can at the same time bind EMC virus. In contrast, glycophorin bound to WGA–Sepharose cannot bind EMC virus in the presence of SDS. The evidence suggests that virus binding to glycophorin-WGA–Sepharose occurred in the absence of SDS because glycophorin was present in aggregated complexes which were large enough either to accommodate both EMC virus and WGA at the same time, or alternatively to provide sufficient attachment sites for multivalent binding of virions.

The attachment of viruses to cells involves a component in the virus and a cellular receptor. The human erythrocyte receptor for the attachment of encephalomyocarditis (EMC) virus is believed to be the major sialoglycoprotein of the cell surface membrane, glycophorin (Enegren & Burness, 1977), which is also probably the receptor for influenza virus and wheat-germ agglutinin (WGA) (Jackson et al., 1973). As with other picornaviruses, the EMC virion component involved in the interaction remains unknown.

Affinity chromatography has proved a useful method to study hormone–receptor interactions (Cuatrecasas, 1974) and the technique has been applied in this laboratory to purify receptors for viruses (Pardoe & Burness, 1980). One of the difficulties frequently encountered in affinity chromatography is finding conditions to elute the bound material. This report describes a novel approach to this problem for EMC virus.

The rationale is as follows: glycophorin attaches to WGA–Sepharose and can be eluted with N-acetylglucosamine (Adair & Kornfeld, 1974; Pardoe & Burness, 1980), the sugar involved in WGA binding to erythrocytes (Burger & Goldberg, 1967). The question raised was whether glycophorin already bound to WGA–Sepharose could simultaneously bind EMC virus and whether the virus would be released together with the glycophorin by N-acetylglucosamine? If so, this would confirm that WGA and EMC virus share a common receptor on the erythrocyte and provide a method to study virus–receptor interactions at one of the simplest levels available.

Attempts to bind EMC virus to glycophorin already attached to a WGA–Sepharose column were first carried out in the absence of SDS. Glycophorin in NaCl–PO₄ (see legend to Fig. 1) was adsorbed to the column followed by adsorption of ³H-labelled EMC virus also in NaCl–PO₄. The column was washed with the same buffer to remove unadsorbed virus and then with N-acetylglucosamine in the buffer to displace the bound glycophorin. About 25% of the virus radioactivity failed to adsorb to the column while 75% became bound but was eluted with N-acetylglucosamine (Fig. 1a); the recovery of radioactivity was 82% of that added to the column. The result suggested that glycophorin bound to WGA–Sepharose also bound EMC virus at the same time and that the virus was displaced with the glycophorin by N-acetylglucosamine.
Fig. 1. Chromatography of glycophorin and EMC virus on WGA-Sepharose. Glycophorin was prepared by the lithium diiodosalicylate procedure of Marchesi & Andrews (1971) and labelled, where indicated, by acetylation with $[^3]H$acetic anhydride (50 mCi/mmol, New England Nuclear) as previously described (Pardoe & Burness, 1980). For chromatographic studies, 1 ml preparations, containing about 230 μg protein (Lowry et al., 1951) or 120 μg sialic acid (Warren, 1959), were added to a 1.5 x 12 cm column of WGA-Sepharose. The column was then washed with 10 ml of the appropriate buffer and, after allowing 30 min for adsorption to take place, with 20 ml buffer to remove unbound material; when an SDS-containing buffer was changed to a non SDS buffer, or vice versa, 100 to 150 ml washes were used. To this column was then added 1 ml EMC virus (17 μg) which had been grown in the presence of $[^35]S$methionine (374 Ci/mmol) or of a $[^3]H$-labelled amino acid mixture (New England Nuclear) and purified as described previously (Burness et al., 1974); adsorption and elution of unbound material was performed as for glycophorin. Finally, material bound to the column was eluted with the appropriate buffer containing 0.1 M-N-acetylglucosamine beginning at the arrow. Flow rates were approx. 0.5 ml/min. (a) Glycophorin followed by $[^3]H$-labelled EMC virus using 0.1 M-NaCl-0.02 M-sodium phosphate buffer pH 8 (NaCl-PO4) throughout. (b) $[^3]H$-labelled EMC virus in the absence of glycophorin. (c) $[^3]S$-labelled glycophorin (broken line) followed by $[^3]S$-labelled EMC virus (continuous line) using NaCl-PO4 containing 0.05% SDS (NaCl-PO4-SDS) throughout. (d) Glycophorin in NaCl-PO4-SDS followed by $[^3]H$-labelled EMC virus in NaCl-PO4. (e) Glycophorin in NaCl-PO4 followed by $[^3]H$-labelled EMC virus in NaCl-PO4-SDS.

An alternative explanation of the result shown in Fig. 1 (a) was that EMC virus became bound to some component on the column other than glycophorin despite the apparent specificity of the elution with N-acetylglucosamine. This possibility was discounted by measuring the binding of $[^3]H$-labelled EMC virus to WGA-Sepharose in the absence of glycophorin. Under these conditions, close to 100% of the radioactivity failed to bind and subsequent elution of the column with N-acetylglucosamine in NaCl-PO4 released no more radioactivity (Fig. 1 b).
Glycophorin binds equally well to WGA-Sepharose in the presence or absence of SDS (Pardoe & Burness, 1980). To determine whether EMC virus binds to glycophorin-WGA-Sepharose in the presence of SDS, $^3$H-labelled glycophorin was adsorbed to WGA-Sepharose in NaCl-PO$_4$-SDS. $^{35}$S-labelled EMC virus in the NaCl-PO$_4$-SDS was then applied to the column which was washed with the buffer to remove unbound material. Close to 100% of the $^{35}$S-labelled EMC virus was not retained by the column while all of the $^3$H-labelled glycophorin previously attached to the WGA-Sepharose remained bound (Fig. 1c). Subsequent washing of the column with N-acetylglucosamine in NaCl-PO$_4$-SDS eluted only $^3$H-labelled glycophorin and no further $^{35}$S-labelled EMC virus. Clearly, although glycophorin bound to WGA-Sepharose in the presence of SDS, EMC virus would not bind to the complex under the same conditions, a result which could have arisen through an effect of the detergent on glycophorin, on the virus or on both.

To test whether the effect was on glycophorin, a preparation was adsorbed to WGA-Sepharose in NaCl-PO$_4$-SDS, followed by extensive washing with NaCl-PO$_4$ to remove the SDS. When $^3$H-labelled EMC virus in NaCl-PO$_4$ was added to the column, close to 100% of the recovered radioactivity failed to bind and subsequent elution of the column with an N-acetylglucosamine solution released no more radioactivity (Fig. 1d); recovery of radioactivity was about 79% of that added to the column. This demonstrated that SDS affected glycophorin in such a way that even after removal of the detergent, at least from the eluting buffer, glycophorin already attached to WGA-Sepharose was unable to serve as a receptor for EMC virus at the same time.

To test whether SDS could also be acting through an effect on the virus, glycophorin was adsorbed to the WGA-Sepharose in the absence of SDS in NaCl-PO$_4$ and this column was tested for its ability to bind $^3$H-labelled EMC virus applied in NaCl-PO$_4$-SDS. About 88% of the recovered radioactivity failed to bind while 12% attached and was subsequently eluted by N-acetylglucosamine (Fig. 1e); recovery was about 78% of the radioactivity added. This failure of virus in SDS to bind to glycophorin-WGA-Sepharose which had not previously been exposed to SDS could be due to a variety of reasons: (i) EMC virus may be unstable in SDS under the conditions used; (ii) the bond formed between EMC virus and glycophorin may involve hydrophobic interactions and, therefore, may be unstable in SDS solutions; (iii) glycophorin and/or the virion attachment component may undergo SDS conformational changes which prevent the two components from interacting. These possibilities were examined as follows. To establish that SDS or some contaminant did not cause breakdown of virus under the conditions we used, $^3$H-labelled EMC virus was kept for 30 min at ambient temperature in NaCl-PO$_4$-SDS and then examined by rate zonal ultracentrifugation on sucrose density gradients also containing NaCl-PO$_4$-SDS. Most of the radioactivity sedimented as a single peak at the position expected for EMC virus, although 18% of the radioactivity was in the pellet at the bottom of the centrifuge tube (Fig. 2a). It was concluded that the failure of EMC virus to bind to glycophorin-WGA-Sepharose in the presence of SDS was not due to virus instability. Our result confirmed an earlier report that EMC virus is stable in SDS (Kerr & Martin, 1972).

To test whether SDS prevented the interaction of EMC virus with its erythrocyte receptor, $^3$H-labelled EMC virus and glycophorin were mixed in NaCl-PO$_4$-SDS and the mixture examined by sucrose density-gradient ultracentrifugation. No peak of radioactivity was detected in the position expected for EMC virus, most of the radioactivity (64%) being found in the pellet at the bottom of the centrifuge tube (Fig. 2a). A similar phenomenon has been noted previously for EMC virus mixed with glycophorin in the absence of SDS and examined by sucrose density-gradient ultracentrifugation (Enegren & Burness, 1977). As membrane glycoproteins are notoriously difficult to keep in solution, it was possible that when a mixture of EMC virus and glycophorin were examined on sucrose gradients, the glycophorin came out...
Fig. 2. Sucrose density-gradient centrifugation studies on EMC virus and glycophorin. (a) $^3$H-labelled EMC virus (17 µg) was incubated for 30 min at ambient temperature in NaCl-PO$_4$-SDS in the presence (O) or absence (•) of glycophorin (230 µg protein), then analysed on 10 to 30% sucrose gradients in NaCl-PO$_4$-SDS in a Beckman-Spinco SW50.1 rotor at 50 000 rev/min for 30 min at 20 °C. P indicates pellet at the bottom of the centrifuge tube. (b) $^3$H-labelled glycophorin (•) mixed with bovine serum albumin (O) in NaCl-PO$_4$-SDS, or $^3$H-labelled glycophorin (■) in NaCl-PO$_4$ analysed in a Beckman-Spinco SW65 rotor at 65 000 rev/min for 5 h on 10 to 30% sucrose gradients made up in NaCl-PO$_4$-SDS or NaCl-PO$_4$, respectively. (c) $^3$H-labelled glycophorin in 0.1% Triton X-100 (■), in 0.5% DOC (□) or in 0.1% SDS (○) analysed in a Beckman-Spinco SW50.1 rotor at 50 000 rev/min for 22.5 h on 10 to 30% sucrose gradients made up in NaCl-PO$_4$ containing the corresponding detergent. Each sample contained bovine serum albumin as a marker but only the u.v. absorbance of that present in the SDS sample is plotted (O). In (b) and (c) results are expressed as fractions along the tube to allow for differences in the number of samples collected per tube resulting from the presence of the various detergents. Sedimentation is from left to right.

of solution causing the virus to co-precipitate. However, this possibility was unlikely since, as shown in Fig. 2(b, c), $^3$H-labelled glycophorin does not precipitate when centrifuged in NaCl-PO$_4$-SDS. The observation that EMC virus and glycophorin centrifuge to the bottom of the tube in NaCl-PO$_4$-SDS suggests, therefore, that the bond between them is stable in the presence of SDS. This observation is interesting in view of the finding that poliovirus is released from complexes with cellular membrane components by SDS (Lonberg-Holm et al., 1975).

To test whether SDS causes conformational changes in the virion attachment component would be premature at present, since the identity of this component of picornaviruses is in dispute. Examination of conformational changes was, therefore, restricted to glycophorin and for this purpose sucrose density-gradient centrifugation was again used. In the absence of SDS, $^3$H-labelled glycophorin sedimented in the form of heterogeneously sized aggregates, about 17% being found in the pellet at the bottom of the tube (Fig. 2b). There were no large aggregates in the presence of SDS and most of the material sedimented more slowly than in the absence of SDS and at a rate similar to that for bovine serum albumin (Fig. 2b). Centrifugation for longer times in SDS showed that $^3$H-labelled glycophorin actually sedimented slightly faster than bovine serum albumin (Fig. 2c), and distinctly faster than glycophorin preparations examined at the same time and under the same conditions except that the SDS was replaced throughout with the detergents sodium deoxycholate (DOC), Triton X-100 (Fig. 2c) or Ammonyx LO (Clough Chemical Co., St. Jean, Quebec, Canada) (result not shown). Using bovine serum albumin as a mol. wt. marker in every tube and following the procedure of Martin & Ames (1961), glycophorin sedimenting in the various detergents was calculated to have the following apparent mol. wt.: 94 500 in SDS; 36 000 in Ammonyx LO; 30 300 in DOC; and 23 700 in Triton X-100. The mol. wt. of glycophorin calculated from its known structure (Marchesi et al., 1976) is approx. 31 000. Thus, allowing for differences in the binding of these various detergents to glycophorin, the results are
consistent with glycophorin being present as monomers in DOC, Triton X-100 and Ammonyx, as dimers or trimers in SDS and as heterogeneous large-sized aggregates in the absence of detergent.

It is deduced from the sucrose density-gradient centrifugation data that glycophorin adsorbed on WGA-Sepharose in the absence of SDS will be in the form of aggregates, while that added in the presence of SDS or on columns subsequently washed with a solution containing SDS is likely to be in a dispersed form, perhaps as dimers or trimers. This perhaps explains why virus can attach to glycophorin-WGA-Sepharose in the absence (Fig. 1a), but not in the presence of SDS (Fig. 1c to e). In the absence of SDS, the multivalent aggregates of glycophorin attached to WGA-Sepharose, being large, are also able to accommodate EMC virus. In the presence of SDS, the glycophorin is in aggregates, perhaps dimers or trimers which are too small to attach to WGA-Sepharose and bind EMC virus at the same time. An alternative explanation is that only the bonds formed between virus and multivalent aggregates are strong enough to retain the virus, whereas the possible divalent and trivalent bonds between virus and dimers and trimers, assuming glycophorin is in one of the forms, are too weak for virus retention.

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Faculty of Medicine
Memorial University of Newfoundland
St. John's, Newfoundland A1B 3V6, Canada

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