PURIFICATION OF VIRUS GLYCOPROTEINS BY AFFINITY CHROMATOGRAPHY USING *LENS CULINARIS* PHYTOHAEMAGGLUTININ

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1. Introduction

The major components of the lipoprotein envelopes of many viruses are glycoproteins [1–5]. These glycoproteins are of considerable interest not only because of their structural and antigenic characteristics but also because their chemical properties may be typical of cell membrane glycoproteins in general. The release of these proteins from purified viruses is commonly achieved by either protease digestion or detergent solubilization [6–8]. However, these procedures frequently cause denaturation or digestion of one or more of the envelope proteins and, furthermore, the separation of solubilized glycoproteins from the non-envelope components of the viruses is often difficult to achieve. Recently, a method was described for the isolation of lymphocyte plasma membrane glycoproteins by means of affinity chromatography of sodium deoxycholate-solubilized membrane on LcH * covalently attached to Sepharose [9]; LcH possesses antibody-like specificity for glucose, mannose and sterically related sugar residues [10]. The present paper describes the application of this technique to the isolation of the envelope glycoproteins of a variety of viruses. The results demonstrate the value of this procedure in the identification and purification of virus glycoproteins and emphasize its general applicability to the separation of cell membrane glycoproteins.

2. Materials and methods

Three strains of influenza virus (X-31 [11], Ao/Bel/42 [12] and Rostock fowl plague) and one parainfluenza virus (Sendai) were used. They were grown in embryonated eggs, and were purified by differential and density gradient centrifugation as previously described [4]. The Sendai virus was a gift from Dr. Michele Henry-Aymard. The mouse mammary tumour virus was derived from spontaneous mammary tumours of C_3H mice, was purified as reported previously [13] and was kindly donated by Dr. Clive Dixon.

Dissociation of virus in 6.6% (w/v) sodium deoxycholate (Fluka AG, Buchs SG, Switzerland) dissolved in 0.1 M Tris-HCl buffer, pH 8.0, was performed as previously described [14]. The suspension was diluted 10-fold and the virus 'cores' were removed by centrifuging at 100,000 *g* for 1 hr. The soluble material was then fractionated using a column of LcH-Sepharose 4B equilibrated with 1% (w/v) sodium deoxycholate in 10 mM Tris-HCl buffer, pH 8.2, as previously reported [9]. Following application of the solution the column was washed extensively with 1% sodium deoxycholate and the adsorbed glycoproteins were subsequently eluted with 2% (w/v) methyl-α-D-mannopyranoside (Fluka AG, Buchs SG, Switzerland) in 1% sodium deoxycholate. Samples of the column effluent were serially diluted in 0.9% NaCl for estimation of the haemagglutinin activity [15] and the neuraminidase activity was determined [16] after brief dialysis against 0.9% NaCl. The unretarded and eluted fractions were precipitated by addition of 2 vol of abs. ethanol at −20°, were recovered by centrifuging and were dissolved in sodium dodecyl sulphate for electrophoresis. The glycolipids of the viral membranes were discarded during this procedure together with the deoxycholate and sugar used for elution. Viral polypeptides were

* LcH: *Lens culinaris* phytohaemagglutinin.

separated and identified by polyacrylamide gel electrophoresis in sodium dodecyl sulphate [4].

3. Results

The Ao/Bel/42 strain of influenza virus was used in initial experiments to determine the efficacy of the separation procedures, primarily because this virus has been clearly shown to contain seven types of polypeptide three of which are envelope glycopeptides [4]. Moreover, two of these glycopeptides are components of the haemagglutinin and the other is a neuraminidase subunit and the biological activities of these glycoproteins are retained following disruption of the virus in deoxycholate. It was, therefore, possible to estimate the recovery of the purified glycoproteins both qualitatively, by polyacrylamide gel electrophoresis and quantitatively in terms of their biological activities.

Table 1 shows that the virus components solubilized in sodium deoxycholate were separated by column chromatography on LcH-Sepharose into two fractions; approx. 93% of the protein applied was recovered. The results also indicate that over 90% of the haemagglutinin and about 88% of the neuraminidase activities were adsorbed to the column and were eluted with methyl-α-D-mannopyranoside. The specific adsorption of the envelope glycoproteins to LcH-Sepharose and their subsequent elution was confirmed by the results shown in fig. 1. Of the seven types of polypeptide seen in the intact virus particles only the three glycopeptides (no. 3, 4 and 6, see fig. 1) were detected in the eluted fraction. These results, therefore, clearly demonstrate the efficient separation of the glycoproteins of this strain of influenza virus.

Table 1
Distribution of protein, haemagglutinin and neuraminidase activities between the unretarded and eluted fractions following chromatography of influenza Ao/Bel/42 virus on LcH-Sepharose.

Fraction	Protein (μg)	Haemagglutinin (total activity)	Neuraminidase (total activity)
Initial	980	8000	180
Unretarded	540	< 50	23
Eluted	375	8000	145

Protein was determined by the method of Lowry et al. [17]. Haemagglutinin activity is expressed as the reciprocal of the dilution causing partial agglutination of a 1% (v/v) suspension of chicken erythrocytes [15]. Neuraminidase activity is expressed as μg of *N*-acetylneuraminic acid released/hr at 37° using fetuin as substrate [16].

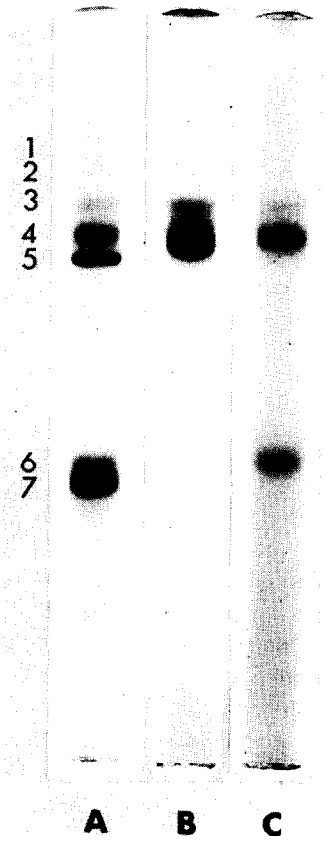

Fig. 1. Polyacrylamide gel electrophoresis patterns of influenza Ao/Bel/42 virus (A and B) and of the fraction specifically adsorbed by LcH-Sepharose and eluted with methyl-α-D-mannopyranoside (C). Electrophoresis was carried out in sodium dodecyl sulphate as previously described [4]. Gels A and C were stained for protein with Coomassie Blue and gel B for carbohydrate with periodate-Schiff reagent [18]. Components no. 3, 4 and 6 of the virus are glycoproteins and were the only components detected in the eluted fraction. Component no. 6 of the virus stained weakly for carbohydrate and was not revealed on gel B even though 4 times as much as that added to gel A was used.

Subsequent experiments indicated that the same procedure can be applied to the isolation of the glycoproteins from a variety of influenza viruses of different antigenic composition. Thus, with both the X-31 strain of Hong Kong influenza virus and Fowl Plague

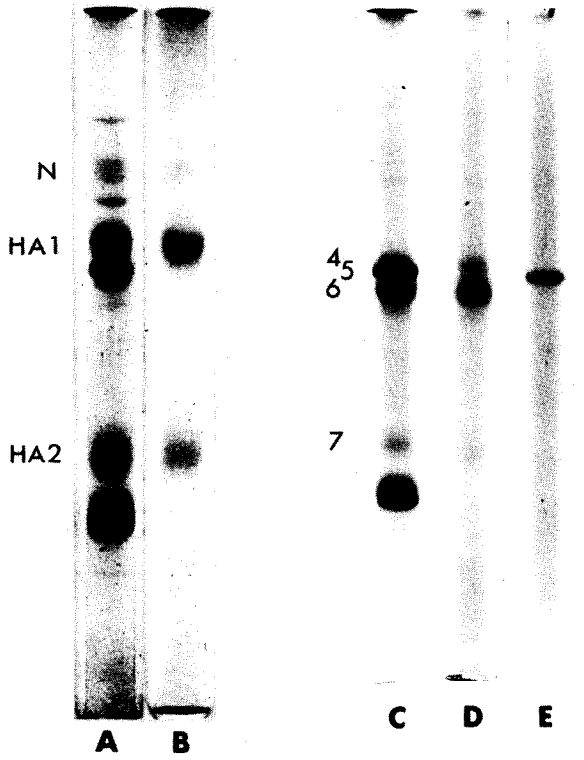

Fig. 2. Polyacrylamide gel electrophoresis patterns of influenza X-31 (A) and Fowl Plague (C) viruses, and of their respective eluted fractions (B and D). Gel E represents the unretarded fraction of Fowl Plague virus. All gels were stained for protein with Coomassie Blue. Components N (gel B) and 7 (gel D) of the eluted fractions stained weakly and can only just be seen in the photograph.

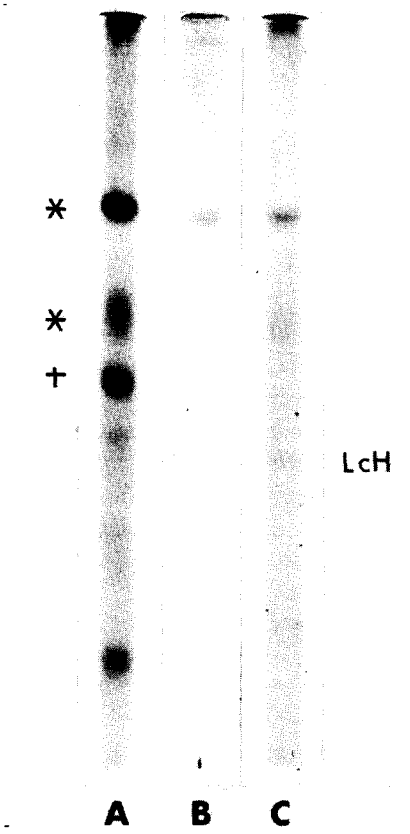

Fig. 3. Polyacrylamide gel electrophoresis patterns of mouse mammary tumour virus (A and B) and of the fraction eluted from LcH-Sepharose (C). Gels A and C were stained for protein with Coomassie Blue and gel B for carbohydrate with periodate-Schiff reagent. The components of the virus indicated (*) stained for both protein and carbohydrate. These glycoproteins are present in the eluted fraction together with some LcH but none of the major non-glycosylated protein of the virus (+) was detected.

virus the unretarded fractions possessed less than 1% of the haemagglutinin activities of the eluted fractions. The fractionation of the polypeptides of these two viruses is illustrated in fig. 2. The neuraminidase and haemagglutinin (components N and HA1 and HA2, respectively; fig. 2) of X-31 are glycoproteins and were the only components detected in the eluted fraction. Fowl Plague virus gave at least eight protein-staining bands of which components 4, 6 and 7 were detected in the eluted fraction (fig. 2). Although only components 6 and 7 gave detectable staining with periodate-Schiff reagent it seems likely, in view of the above results that component 4 is also a glycoprotein. As shown in fig. 2 the unretarded fraction of Fowl Plague virus contained primarily component 5 and may represent a convenient source for this component (viral polypeptides not present in the unretarded or eluted fractions were separated by centrifuging prior to the addition of the solubilized virus to the LcH-Sepharose column).

In view of the success achieved with the above viruses, attempts were next made to determine whether the procedure is generally applicable to the isolation of the glycoproteins of enveloped viruses. Mouse mammary tumour virus and the parainfluenza Sendai virus were chosen for investigation as representative of their groups because their polypeptide compositions are known ([19, 20]; [3] and M. Henry-Aymard and J.J. Skehel, unpublished observations, respectively). As shown in fig. 3 the two glycoproteins of the mammary

tumour virus were specifically adsorbed to the LcH-Sepharose and were thereby separated from the non-glycosylated virus proteins. In this case, however, the eluted material was contaminated with a small amount of LcH. The two glycoproteins of Sendai virus were also separated by this procedure; these results will be published elsewhere.

4. Discussion

The dissociation of enveloped viruses by sodium deoxycholate and the characterization of the components have been reported previously [14, 21, 22]. The main advantage of sodium deoxycholate is that the antigenic and enzymic activities of the envelope proteins survive the disruption of the virus. The present results indicate that this dissociation procedure can be combined with affinity chromatography on LcH-Sepharose to give an efficient procedure for the separation and identification of virus envelope glycoproteins. Although, when colums of fresh LcH-Sepharose were used, the eluted fraction contained small amounts of LcH (fig. 3) this contaminant can be readily removed by adsorption with a little Sephadex G25. These results reinforce the previous proposition [9] that due to the similar carbohydrate compositions of glycoproteins and to the fairly broad specificity of LcH for carbohydrates, the method is of general applicability to the isolation of membrane glycoproteins. The method may also be of value for the removal of glycoprotein impurities from non-glycosylated proteins.

In conclusion, it is suggested that since the development of immunity against many virus infections involves the formation of antibodies against the virus envelope glycoproteins, the procedures described here may be of value in the preparation of purified subunit vaccines.

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