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BOVINE VIRAL DIARRHEA VIRUS: PURIFICATION OF SURFACE PROTEINS IN DETERGENT-CONTAINING BUFFERS BY FAST PROTEIN LIQUID CHROMATOGRAPHY

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SUMMARY

Bovine viral diarrhea virus was purified by lectin chromatography. The glycoprotein peplomers were dissociated from the virion by treatment with detergent. By a second lectin gel chromatography the glycoconjugates containing terminal galactose were prepared. In combination with lectin affinity chromatography, ion-exchange chromatography on Mono-Q in the presence of the low-UV-absorbing detergent Berol 172 proved to be a powerful technique both for analytical and preparative applications.

INTRODUCTION

Bovine viral diarrhea virus (BVDV) or bovine mucosal disease virus is a RNA virus of the genus *Pestivirus*, family *Togaviridae*. It is an enveloped virus with peplomers embedded in the lipid layer which loses most of its infectivity when purified by conventional techniques, *e.g.*, gradient centrifugation. Apparently the peplomers, of which some are glycoconjugates, are very sensitive to mechanical stress and easily dissociate from the viron. This fact suggests that they may have a short, hydrophobic part, serving as an anchor in the envelope. By use of affinity chromatography on Sepharose 2B, coupled with the galactose-specific lectin of *Crotalaria juncea* (sunn hemp), the virus was prepared with a recovery of 85% of the original infectivity[1].

The peplomers were dissociated from the virion by treatment with detergent and rechromatographed on a second *Crotalaria* lectin column. In this way it was possible to isolate the glycoconjugates carrying terminal galactose. Two components of viral origin and one also present in uninfected culture medium were detected by immunoelectrophoresis[2].

For further purification and analysis of the proteins, fast protein liquid chro-

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matography (FPLC) seemed to be an attractive method. However, the UV absorption of most detergents in combination with the tendency of the material to aggregate and clog the columns in the absence of detergent is a severe problem when working with hydrophobic proteins. The introduction of the low-UV-absorbing detergent Berol 172 facilitated the use of FPLC in the investigation of the surface proteins of BVDV.

MATERIALS AND METHODS

Preparation of viral proteins

BVDV of strain Ug-59 was cultured on fetal calf turbinate cell in Eagles MEM, but otherwise as described earlier[1].

Affinity chromatography I

After centrifugation of the culture medium the supernatant was chromatographed on a *Crotalaria juncea* lectin column equilibrated in PBS (phosphate buffered saline, 0.02 *M* sodium phosphate, pH 7.4, containing 0.15 *M* sodium chloride)[1]. Unadsorbed material, designated C-I:1 (C-I = first *Crotalaria* lectin chromatography; 1 = unadsorbed material), was not further investigated. Adsorbed material was eluted with 5% lactose and the pooled UV-absorbing fractions were designated C-I:2 (2 = adsorbed material). This material was treated with 1% Berol 172 to dissociate the peplomers from the virion. Lactose was removed by dialysis against 0.05 *M* Tris–HCl pH 7.0, 0.2% Berol or by gel filtration on Sephadex G-25 in the same buffer.

Affinity chromatography II

The lactose-free viral mixture was chromatographed on a second *Crotalaria* lectin column. Unadsorbed material and material desorbed with 5% lactose were designated C-II:1 and C-II:2, respectively. Further details of the preparation were described elsewhere[2,3].

Blank experiment

Uninfected culture medium was treated exactly as the infected, and C-II:1u and C-II:2u fractions were collected.

Ion-exchange chromatography (FPLC)

Analytical and preparative chromatography of the fractions mentioned above were performed on Mono-Q columns with the Pharmacia FPLC system, consisting of a GP-250 Gradient Programmer, two P-100 pumps, a FRAC-100 fraction collector, a UV-1 monitor with a HR flow cell, a 10-ml Superloop and a REC-482 two-channel recorder. Two buffer systems were used: (1) 0.05 *M* Tris–HCl, pH 7.0; (2) 0.02 *M* piperazine–acetic acid (pH 6.0); both with the addition of 1 *M* sodium chloride in the final buffer and, if not otherwise stated, 0.18–0.20% (w/v) Berol in all buffers. The flow-rate was 2 ml/min. In preparative chromatography the samples were applied to the columns with the 10-ml or 50-ml Superloop supplied with the chromatography system. Before sample application, 4 ml of the starting buffer were passed through the column.

In order to investigate the possible appearance of artifacts due to micelle for-

mation, blank experiments were performed in 0.05 *M* Tris-HCl (pH 7.0), containing 0.2% Berol 172, and with 2 *M* sodium chloride in the final buffer (Fig. 1A). Other blank experiments were performed in the same buffer system but containing 1% detergent and with 1 *M* sodium chloride in the final buffer (Fig. 1B).

Fractions eluted by the pH 7 buffer system, which has a poor buffer capacity, were easily transformed to pH 6 by adding two volumes of the pH 6 buffer.

Berol 172

Berol 172 (Berol Scandinavia, Stenungsund, Sweden) is a non-ionic detergent with low absorption in the UV region. It is a condensation product of *n*-alcohols with chain lengths C_{10}/C_{12} and ethylene oxide. The solubilizing properties of the detergent are similar to those of Triton X-100 and Tween-20. The critical micelle concentration lies between 0.2 and 0.5%, depending on the physical and chemical environment. In this study, Berol 172 was used in 1% concentration to dissociate the surface proteins from the virion, and in 0.2% concentration in all buffers to keep the proteins in solution. A very similar detergent, Berol 185, is also available.

RESULTS AND DISCUSSION

Investigation of micelle formation

In the blank experiments with 0.2% Berol an absorbancy shift from baseline level to 1.1 was registered at a salt concentration of 1.8 *M* (Fig. 1A). The explanation for this phenomenon is probably a change in refractive index due to micelle formation, because the absorbancy was low in all fractions when measured on a spectro-

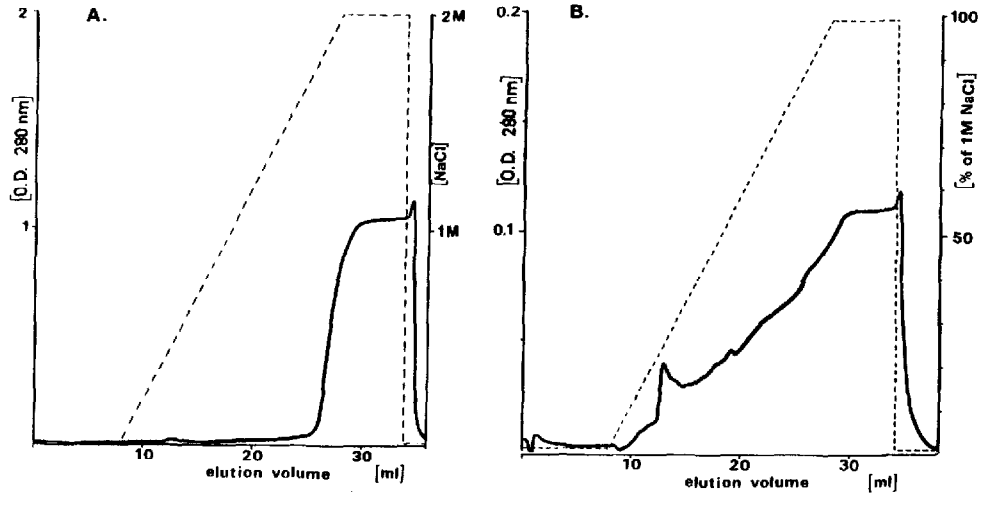

Fig. 1. FPLC on Mono-Q. A, Blank determination with 0.05 *M* Tris-HCl, pH 7, 0.2% Berol 172 and with 2 *M* sodium chloride in the final buffer. The CMC is reached at 1.8 *M* salt. B, Blank determination above the CMC in 0.05 *M* Tris-HCl (pH 7)/1% Berol 172 and with 1 *M* sodium chloride in the final buffer. The CMC is reached even in the absence of salt at this detergent concentration. The micelle concentration increases along the salt gradient.

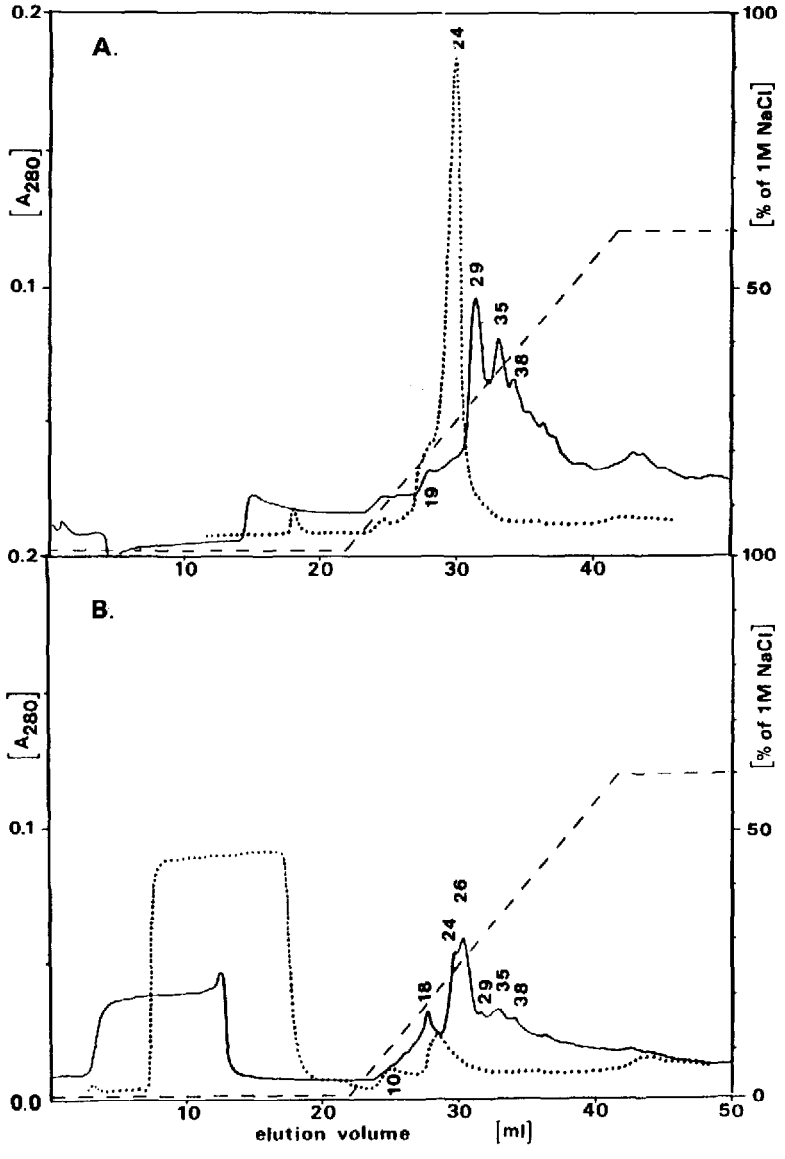

Fig. 2. FPLC on Mono-Q with 0.05 *M* Tris–HCl (pH 7)/0.2% Berol 172. A, Comparison of uninfected (dotted line) and infected C-II:2 material. Sample volumes of 10 ml were applied with the Superloop. Infected material was eluted above 28% of the 1 *M* sodium chloride concentration. The main peak in an uninfected preparation eluted by 24% of the 1 *M* sodium chloride is not visible in the infected material. B, Comparison of uninfected (dotted line) and infected C-II:1 material. Proteins of infected fluid were eluted by 24 and 26% of the 1 *M* sodium chloride, while only small quantities of uninfected material were retarded on the column.

photometer. Apparently the critical micelle concentration (CMC) is exceeded during the experiment. The fact that an increased salt concentration will increase the hydrophobic interaction supports this theory. Accordingly, experiments performed as

indicated with different buffers and detergent concentrations should be an excellent method for determining CMC values. To answer the question whether a column is needed at all, experiments without any column attached to the system were run and gave similar results.

In the blank experiments with a detergent concentration well above CMC the baseline follows the salt gradient as could be expected (Fig. 1B). The concentration of micelles increases with the salt concentration while the fraction of free detergent decreases.

Thus we conclude that ion-exchange chromatography on Mono-Q and Mono-S should be performed below the CMC of the detergent to avoid the artifacts appearing when the CMC is exceeded. If this detergent concentration is too low to keep the proteins in solution the entire elution has to be performed above the CMC but in this case a sloping baseline has to be accepted.

FPLC of viral material

Material passing through the second lectin column (C-II:1) was chromatographed on a Mono-Q column and compared to uninfected material (C-II:1u), treated in the same way (Fig. 2B). Nearly all material from uninfected fluid passed through the column unretarded, while components from infected culture medium showed peaks at 18, 24 and 26% of the 1 *M* sodium chloride. The sample volume in both cases was 10 ml.

When material desorbed from the second lectin column (C-II:2) was compared to uninfected material (C-II:2u) in a similar way no material passed the column (Fig. 2A). Uninfected material showed a main peak at 24% of the 1 *M* sodium chloride and shoulders at 17 and 19%. Infected material, on the other hand, was eluted mainly at 29, 35 and 38% with minor peaks at 41 and 44%.

It appears that the peaks at 17, 19 and 24% and the main part of the material which passes the column unretarded are connected with the uninfected culture medium. Material corresponding to salt concentrations above 28% of the 1 *M* solution seems to be of viral origin. Some authors have reported the existence of three[4] or four [5] viral surface components as analyzed by sodium dodecyl sulphate electrophoresis. We are as yet unable to correlate those results to ours.

Increase of resolution

When fractions containing material eluted mainly at 24 and 29% of 1 *M* sodium chloride were pooled from several runs and rechromatographed on the Mono-Q column the pattern of Fig. 3A was obtained. Two ways to increase the resolution were tested: (1) one part of the material of the main double peak (Fig. 3A) was rechromatographed in buffer system 1 but with a combination of stepwise and continuous elution (Fig. 3C); (2) another part of the same material was diluted in two volumes of piperazine buffer, pH 6, and chromatographed in buffer system 2 (Fig. 3B). Using method 2 a remarkable increase in resolution was obtained and the two components were eluted at 24 and 91%. When the peaks of the stepwise elution were tested by the second method this result was confirmed (Fig. 3D, E). This experiment also showed that the stepwise elution actually gave a good resolution although the peaks were not as high and narrow.

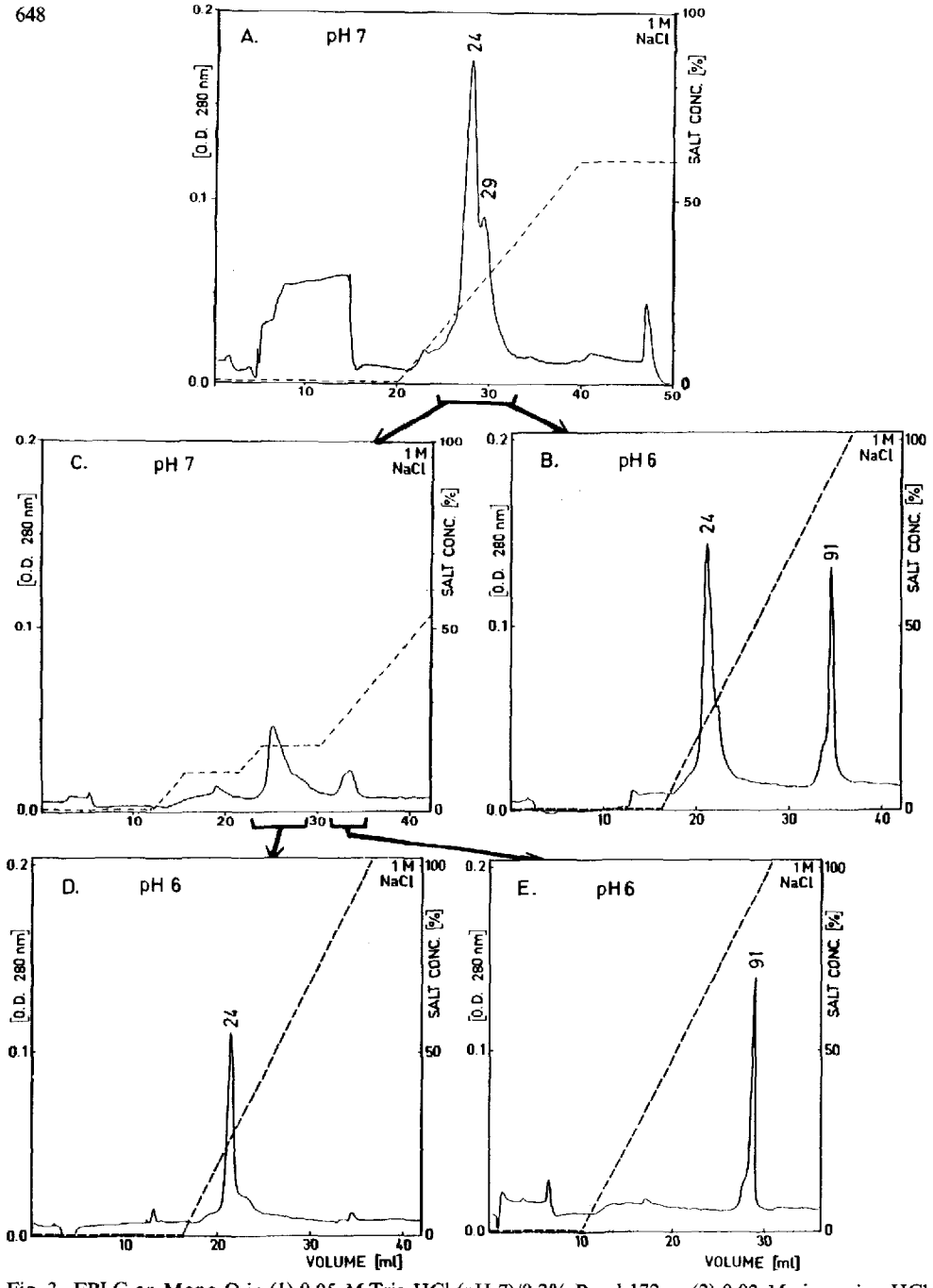

Fig. 3. FPLC on Mono-Q in (1) 0.05 *M* Tris–HCl (pH 7)/0.2% Berol 172 or (2) 0.02 *M* piperazine–HCl (pH 6)/0.2% Berol 172. A, Chromatography at pH 7 of pooled fractions from earlier runs, containing material which was eluted mainly at 24 and 29%. B, One part of the material from the entire double peak was diluted in twice the volume of pH 6 buffer. Due to the weak buffer capacity of the pH 7 buffer, pH 6 was obtained in the sample. Chromatography on Mono-Q equilibrated at pH 6 gave a good resolution. C, Another part of the same material was diluted with an equal volume of the starting buffer, pH 7, to decrease the ionic strength and rechromatographed. By holding the gradient at 12 and 18% the resolution was improved. D, The material of the first peak obtained by stepwise elution at pH 7 was converted into pH 6 by dilution and chromatographed on Mono-Q at pH 6. E, The material of the second peak obtained by stepwise elution at pH 7 was converted into pH 6 and chromatographed on Mono-Q at pH 6.

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