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Detergent extraction of herpes simplex virus type 1 glycoprotein D by zwitterionic and non-ionic detergents and purification by ion-exchange high-performance liquid chromatography

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Abstract

Detergents (surfactants) are the key reagents in the extraction and purification of integral membrane proteins. Zwitterionic and non-ionic detergents were used for the extraction of recombinant glycoprotein D (gD-1) of herpes simplex virus type 1 (HSV-1) from insect cells infected with recombinant baculovirus. The highest yield was obtained with the two alkyl carboxybetaine detergents (*N*-dodecyl-*N*,*N*-dimethylammonio)undecanoate [DDMAU, critical micelle concentration (CMC)=0.13 m*M*] and (*N*-dodecyl-*N*,*N*-dimethylammonio)butyrate (DDMAB, CMC=4.3 m*M*). Therefore these zwitterionic detergents were used as additives to the elution buffers in ion-exchange high-performance liquid chromatography (HPIEC) to purify gD-1 of HSV-1 from the extracts. The non-ionic detergent pentaethyleneglycol monodecyl ether ($C_{10}E_5$) that was used in earlier studies [R.A. Damhof, M. Feijlbrief, S. Welling-Wester, G.W. Welling, J. Chromatogr. A, 676 (1994) 43] was used for comparison. Two columns were used, Mono Q and Resource Q, at 1 and 5 ml/min flow-rates, respectively. The results show that the detergents DDMAU and $C_{10}E_5$ are superior to DDMAB, when the detergents were used as additives to the elution buffers at 0.2% (w/v). With 0.2% DDMAB in the eluent, purification of HSV gD-1 was not possible. Detergents with a high CMC may be less suitable as additives in elution buffers. HPIEC at flow-rates of 1 and at 5 ml/min showed satisfactory results. At 5 ml/min HSV gD-1 was mainly concentrated in two eluent fractions. The highest recovery of gD-1 was obtained either by chromatography of a $C_{10}E_5$ extract using a Mono Q column at a flow-rate of 1 ml/min or by chromatography of a DDMAU extract using a Resource Q column at a flow-rate of 5 ml/min.

Keywords: Mobile phase composition; Surfactants; Glycoproteins; Proteins; Membrane proteins

1. Introduction

Detergents (surfactants) are the key reagents in the extraction and purification of integral membrane proteins [1]. Solubilization of membranes including its proteins or selective extraction by detergents is often the first step in the purification of an integral membrane protein.

Detergents are lipid-like substances. Like the major constituent of the membrane, the phospholipid molecule, they contain a hydrophilic head and a hydrophobic tail. They are able to compete with the lipids in a bilayer and are more hydrophilic than the lipids. As a consequence, detergent–protein complexes are soluble in aqueous solutions, and the detergent molecules, in mimicking the lipid molecules, help to maintain the native configuration of the membrane proteins during a purification procedure.

There are several categories of detergents [1–10]: (a) ionic detergents e.g., sodium dodecyl sulfate

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(SDS), (b) bile salts, which are mild ionic naturally occurring detergents, e.g., cholate, taurodeoxycholate, (c) mild non-ionic detergents and (d) mild amphoteric detergents. Detergents of categories c and d are particularly relevant for extraction and purification by ion-exchange high-performance liquid chromatography (HPIEC) and they are listed in Table 1 together with their critical micelle concentration (CMC).

The choice of a suitable detergent may depend on several factors, i.e., CMC, hydrophile–lipophile balance number (HLB), micellar molecular mass, cloud point, UV-transparency, effect on biological activity and price.

In the present study we will focus on the CMC. The CMC is the concentration of monomer at which micelles i.e., spherical bilayer aggregates of detergent molecules, begin to form. Triton X-100 has a low CMC, 0.24–0.30 m*M*, and is difficult to remove by dialysis. Octylglucoside has a high CMC, 25 m*M*, and can easily be removed by dialysis. Therefore, further studies to be carried out with a particular membrane protein may determine the choice of detergent. Some studies require a soluble protein–detergent complex in order to maintain biological activity. In such cases the CMC is of less importance, although the relatively high concentration of detergent present in extracts may affect the biological activity to some extent. Similarly, high concentrations of certain detergents may interfere with immunological assays e.g., an enzyme-linked immunosorbent assay (ELISA).

In earlier studies, we used the integral membrane proteins of different viruses as a model for the development of methodologies for the purification of membrane proteins with different detergents and different modes of high-performance liquid chromatography (HPLC) [8,12–14]. This resulted in a two-step elution protocol with a non-ionic detergent at low and high concentration in the eluent for HPIEC [15–17].

In the present study, a number of non-ionic and

Table 1
CMC of non-ionic and amphoteric detergents

Detergent	Description	CMC (m*M*)
Non-ionic		
Triton X-100	*tert*.-$C_8\phi E_{9.6}$	0.24–0.30
Nonidet-P40	*tert*.-$C_8\phi E_9$	0.29
Triton X-114	*tert*.-$C_8\phi E_{7-8}$	0.20
Penta-ethyleneglycol monodecyl ether	$C_{10}E_5$	0.69
Penta-ethyleneglycol monododecyl ether	$C_{12}E_5$	0.049
Emulphogen BC-720	$C_{12}E_8$	0.08
Lubrol PX	$C_{12}E_{9-10}$	0.02–0.1
Thesit	$C_{12}E_9$	<0.1
Brij 35	$C_{12}E_{23}$	0.091
Tween 80	$C_{18:1}$ sorbitan E_{20}	0.012
Octylglucoside	C_8 glycoside	25.0
Dodecyl-β-D-maltoside	C_{12} maltoside	0.20
Hecameg	6-*O*-(*N*-Heptylcarbamoyl)-methyl-O-D-glucopyranoside	19.5
Mega-10	*N*-(D-Gluco-2,3,4,5,6-penta-hydroxyhexyl)-*N*-methyldecanamide	6.2
Amphoteric		
3-[Cholamidopropyl)dimethylamino]-1-propanesulfonate (CHAPS)	Bile acid derivative	4–6
Zwittergent 3-12 (sulfobetain SB 3-12)	Sulfopropylammonium compound	3.6
(*N*-Dodecyl-*N*,*N*-dimethylammonio)undecanoate (DDMAU)	Alkyl carboxybetaine	0.13
(*N*-Dodecyl-*N*,*N*-dimethylammonio)butyrate (DDMAB)	Alkyl carboxybetaine	4.3
Dodecyl dimethylamineoxide	$[C_{12}N^+(CH_3)_2O^-]$ (above pH 7)	2.2

Data are from Refs. [1–11]; C_xE_y, *x* refers to the number of C atoms in the alkyl chain and *y* to the average number of oxyethylene units; a phenyl ring is designated by φ; *tert*.-C_8 refers to a tertiary octyl group and $C_{18:1}$ indicates an 18-carbon chain with one double bond.

amphoteric detergents will be compared with respect to the extraction of a recombinant integral membrane protein i.e., glycoprotein D of herpes simplex virus type 1 (gD-1) [15,18–22] from cells infected with recombinant baculovirus. Subsequently, two detergents with a large difference in CMC will be compared with regard to their suitability as additive to the eluents for HPIEC using either a Mono Q or a Resource Q column. The two detergents are the alkylcarboxybetaine compounds, (*N*-dodecyl-*N*,*N*-dimethylammonio)undecanoate (DDMAU, CMC= 0.13 m*M*) and (*N*-dodecyl-*N*,*N*-dimethylammonio)-butyrate (DDMAB, CMC=4.3 m*M*) [10,23].

2. Experimental

2.1. HPIEC

Chromatography was performed with a system consisting of an LKB Model 2150 pump (Pharmacia Biotech, Roosendaal, Netherlands), a Rheodyne (Inacom, Veenendaal, Netherlands) Model 7125 injector and a Waters Model 441 detector (Millipore–Waters, Etten-Leur, Netherlands). HPIEC was performed with either a Mono Q HR 5/5 column (50 mm×5 mm I.D.) (Pharmacia Biotech) or a Resource Q column (30 mm×6.4 mm I.D.) (Pharmacia Biotech). The flow-rate was 1 ml/min when the Mono Q column was used and 1 ml/min or 5 ml/min when the Resource Q column was used (see below). The samples (500 μl, containing 10–12 mg protein) of the infected cell extracts [containing 1% (w/v) of detergent] were centrifuged at 14 000 *g* at 4°C for 5 min and diluted with 20 m*M* Tris–HCl, pH 7.8 (buffer A), to a final detergent concentration of 0.01%, prior to application to the column. After sample application, the column was washed in several steps. The first wash step was isocratic elution for 15 min with buffer A. A second wash step was elution for 10 min with buffer B (20 m*M* Tris–HCl, pH 7.8 containing 0.5 *M* NaCl). The third wash step was isocratic elution for 15 min using buffer A to remove the salt. The fourth step was equilibration of the column with 20 m*M* Tris–HCl, pH 7.8, containing 0.2% detergent (buffer C), for 12 min. The same detergent was added to the eluent as was used for the extraction. The fifth step involved elution of the membrane proteins. This was performed by a 12-min linear sodium chloride gradient from buffer C to 0.5 *M* NaCl in the same buffer (buffer D). The detergents used in this study were $C_{10}E_5$ (Kwant-Hoog Vacolie Recycling and Synthesis, Bedum, Netherlands), DDMAU and DDMAB (both of Calbiochem-Novabiochem, La Jolla, CA, USA). When the chromatography was performed with a Mono Q HR 5/5 column all five steps were performed at a flow-rate of 1 ml/min. When the Resource Q was used, two different protocols for chromatography were used. In the first protocol, steps 1, 2 and 3 were performed at a flow-rate of 5 ml/min, in 3, 2 and 3 min, respectively. To allow comparison with HPIEC on the Mono Q, steps 4 and 5 were performed at a flow-rate of 1 ml/min. In the second protocol all five steps were performed at a flow-rate of 5 ml/min, while steps 4 and 5 were reduced to 3 min each. The absorbance was monitored at 280 nm.

Fractions of 5 ml were collected during steps 1 to 4 and fractions of 2 ml were collected during gradient elution of step 5. Fractions of steps 1 to 4 were dialyzed and lyophilized before analysis. Fractions of step 5 were analyzed directly. Fractions were analyzed by SDS–polyacrylamide gel electrophoresis (PAGE) and ELISA.

2.2. SDS–PAGE

Dialyzed and lyophilized samples (125 μl) of selected HPIEC fractions were analyzed by SDS–PAGE on 12.5% gels under reducing conditions [24]. After electrophoresis, gels were fixed and silver stained as described [25].

2.3. ELISA

Microtiter plates were coated for 18 h at 4°C with serial dilutions (in 50 m*M* $NaHCO_3$ buffer, pH 9.6) of samples of the collected fractions. After washing with phosphate-buffered saline (PBS), pH 7.4 containing 1 *M* NaCl and 0.3% Tween-20, plates were incubated with 1:6400 diluted monoclonal antibody (mAb) HD1 for 1 h. The mAb HD1 is directed against gD-1 and gD-2 and conformation-dependent [26]. After washing, plates were incubated for 1 h at 37°C with peroxidase-labeled sheep anti-mouse IgG

(Sanofi Diagnostics Pasteur, Marnes-la-Coquette, France). After color development with *o*-phenylenediamine dihydrochloride, the optical density was measured at 492 nm. Glycoprotein D-1 concentrations were calculated at $OD_{492}=1.2$ by using a gD-2 standard in combination with amino acid analysis.

2.4. Extraction of recombinant gD-1 from Sf21 cells using non-ionic detergent $C_{10}E_5$, DDMAU, DDMAB, octylglucoside, Hecameg, dodecyl-β-D-maltoside

Sf21 cells were grown in protein-free insect cell culture medium (Insect X-press, Bio-Whittaker, Walkersville, MD, USA) containing 10 μg/ml gentamicin. Insect cells ($2.5\cdot10^8$) were infected at a multiplicity of infection of 5 plaque-forming-units per cell by recombinant baculovirus containing the gD-1 gene (designated as gD-1-baculovirus). After four days of infection at 27°C, cells were collected by centrifugation (100 *g*, 10 min, room temperature) and washed three times in ice-cold PBS. For extraction of membrane proteins, the cell pellet ($5\cdot10^7$ cells per ml) was resuspended in ice-cold 20 m*M* Tris–HCl, pH 7.8, 2 m*M* phenylmethylsulfonyl fluoride (PMSF), 1 m*M* tosyllysine chloromethyl ketone (TLCK) and subsequently an equal volume of the same buffer was added, containing 2% (w/v) $C_{10}E_5$, 2% DDMAU, 2% DDMAB, 2% octyl-glucoside (Boehringer Mannheim, Almere, Netherlands), 2% Hecameg (Vegatec, Villejuif, France) and 2% dodecyl-β-D-maltoside (Sigma, Brunschwig Chemie, Amsterdam, Netherlands), respectively. The cell suspension in the detergent solution (final detergent concentration 1%) was incubated on ice for 1 h. Cell debris was removed by low-speed centrifugation (10 min, 2000 *g*). The supernatants (extracts) after ultracentrifugation (70 000 *g*, 1 h, 4°C) contain gD-1 and were stored in aliquots at −80°C. The amounts of gD-1 in the extracts were quantitated by ELISA.

3. Results and discussion

3.1. Extraction of recombinant gD-1 with different detergents

Extraction of membrane proteins from infected cells or from virus particles is often the first step in the purification of membrane proteins. The suitability of different detergents to extract recombinant-gD-1 from insect cells infected with gD-1-baculovirus was investigated. The amount of gD-1 extracted was determined by ELISA. The yields together with the characteristics of the detergents are given in Table 2. Extraction of infected cells with 1% $C_{10}E_5$, 1% dodecyl-β-D-maltoside, 1% DDMAU and 1% DDMAB, respectively, yielded approximately similar amounts of gD-1. Low yields were obtained by extraction with detergents, having a high CMC. A subsequent, second extraction of the infected cells with a higher concentration (a final concentration of 2% detergent) of these detergents with a relatively high CMC, e.g., Hecameg and octylglucoside, greatly enhanced the yields of gD-1 (data not shown). In an earlier study [8], in which a number of polyoxyethylene alkylethers were compared, it was shown that the highest yields were obtained between HLB values of 11.5 to 12.5. The CMC of these detergents seemed to be of less importance, although in that particular study the yield of Sendai virus membrane proteins after extraction with octylglucoside (CMC 7.1 mg/ml; HLB 12.6) was only 50% of that obtained with $C_{10}E_5$.

3.2. HPIEC of detergent extracts containing the HSV membrane protein gD-1

In previous studies a multi-step purification strategy was developed for the purification of integral membrane proteins from different sources, Sendai virus [17], *Plasmodium falciparum* [16] and herpes simplex virus [15]. The basic principle was a sodium chloride gradient elution with eluents without detergent, followed by a second elution (a blank run)

Table 2
Yields of recombinant gD-1 after extraction of cells infected with gD-1-recombinant baculovirus with different detergents

Detergent	CMC (m*M*)	Yield gD-1 (mg)
$C_{10}E_5$	0.69	24.3
Octylglucoside	25.0	1.1
Dodecyl-β-D-maltoside	0.20	23.0
Hecameg	19.5	2.6
DDMAU	0.13	37.8
DDMAB	4.3	37.3

with a sodium chloride gradient with buffers containing 0.1% detergent. During elution with buffers without detergent, the hydrophilic proteins were eluted and during the blank run with buffers containing detergent, the hydrophobic membrane proteins were eluted. In this way a selective elution of membrane proteins could be achieved.

Due to several wash steps, the whole procedure is rather time consuming. Therefore we investigated in the present study whether we could apply the above-mentioned principle in a fast chromatographic procedure. For this we used a Resource Q column, which allows a higher flow-rate than the Mono Q column. The detergents DDMAB and DDMAU, which showed promising results in the extraction of gD-1, were investigated as additives to the elution buffers. The non-ionic detergent $C_{10}E_5$ which was used in an earlier study [15] was included for comparison. Samples of the detergent extracts, containing an equal amount of gD (approximately 1.2 mg of gD-1), were subjected to the five consecutive HPIEC steps. Approximately 10 to 15% of the total amount of protein in the extract is gD-1. HPIEC was performed with 0.2% of the same detergent as used for the extraction, added to the elution buffer for step 5. The CMC values of the three detergents studied, i.e., $C_{10}E_5$, DDMAU and DDMAB, are 0.026%, 0.005% and 0.128%, respectively. Fractions were collected during the chromatographic steps and analyzed for the presence of gD-1. Table 3 summarizes the different procedures used with respect to detergents, columns, flow-rates and gD-1 recovery. Glycoprotein D-1 was mainly eluted during the sodium chloride gradient in the presence of 0.2% detergent in the eluent (step 5). No gD-1 was found in the flow-through fractions, and the fractions of the equilibration step (step 4) with buffer C. Glycoprotein D-1 was only found in the fractions eluted with buffer B (step 2). When the $C_{10}E_5$ and the DDMAU extracts were used as starting material for the chromatography approximately 15% of the gD-1 applied to the column was eluted together with other proteins in step 2 (the sodium chloride wash step without detergent). When the DDMAB extract was applied to the columns either no gD-1, or only trace amounts could be detected among the other proteins that were eluted. An explanation for this could be that in the case of the $C_{10}E_5$ and the DDMAU extract, detergent molecules are still attached to gD-1 bound to the column after sample application and washing with buffer A, resulting in partial elution of gD-1. Due to the higher CMC of DDMAB, this detergent probably is more easily removed during and after sample application with the result that gD-1

Table 3
Chromatographic conditions and gD-1 recovery

Detergent in eluent	Column	Flow-rates steps 1,2,3[a] (ml/min)	Flow-rates steps 4,5[a] (ml/min)	Recovery gD-1[b] (%)
$C_{10}E_5$	Mono Q	1	1	72
	Resource Q	5	1	34
	Resource Q	5	5	50
DDMAU	Mono Q	1	1	15
	Resource Q[d]	5	1	34
	Resource Q[f]	5	5	72
DDMAB	Mono Q	1	1	nd[c]
	Resource Q[e]	5	1	20[g]
	Resource	5	5	nd

[a] See Section 2.1.
[b] Expressed as the percentage of the amount applied to the column.
[c] nd=Not determined.
[d] See Fig. 1a Fig. 1d.
[e] See Fig. 1b Fig. 1e.
[f] See Fig. 2.
[g] Not purified.

is not eluted, or only trace amounts are eluted during the sodium chloride wash step (step 2).

To compare the Mono Q and the Resource Q column with respect to the purification of gD-1, protocol 1 as described in Section 2.1 was used. Briefly, this implied that the wash steps were performed at a flow-rate of 5 ml/min for the Resource Q column and at 1 ml/min for the Mono Q. The flow-rate (1 ml/min) during the sodium chloride gradient in the presence of the detergent was identical for the two columns. The elution patterns using the Resource Q column of the sodium chloride gradients in the presence of the detergents DDMAU and DDMAB (step 5, protocol 1) are shown in Fig. 1a Fig. 1b, respectively, together with the concentration (μg/ml) of gD-1 in each fraction as determined by ELISA. The corresponding SDS gels of the fractions are shown in Fig. 1c and d.

Fig. 1c shows that fractions 4 and 5 contain mainly three polypeptide bands with molecular mass (M_r) of 54 000–52 000, 37 000 and 22 000, corresponding to gD-1, and fragments thereof. This was confirmed by immunoblotting (data not shown) and ELISA analysis of the fractions with a gD-specific

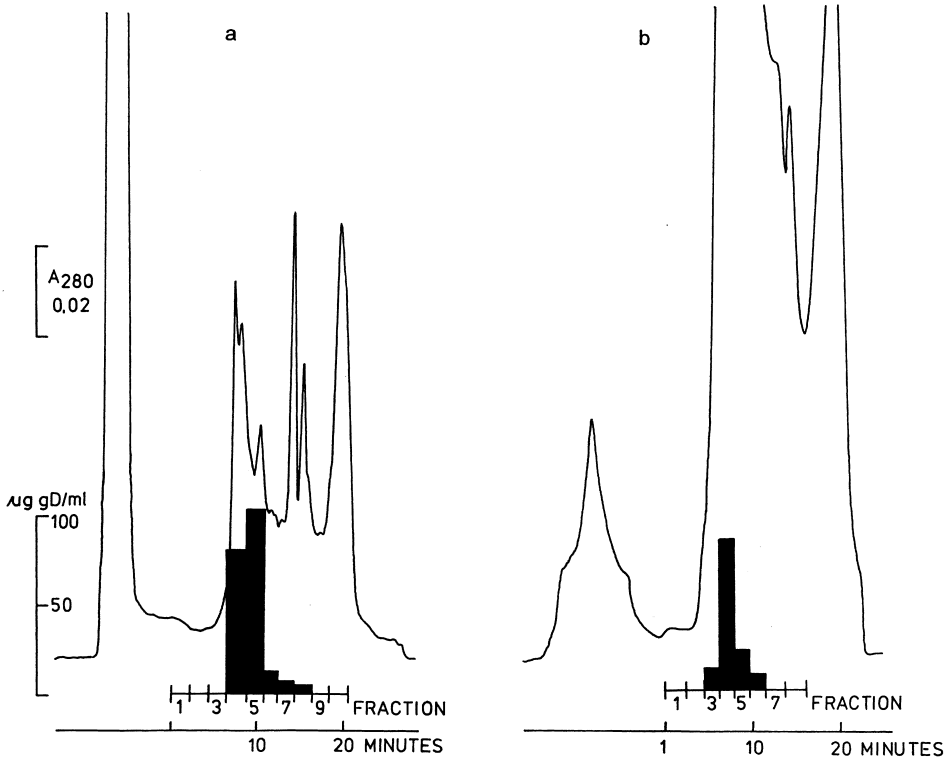

Fig. 1. HPIEC elution profile of a DDMAU extract (a) and a DDMAB extract (b) of insect cells infected with a recombinant gD-1-baculovirus. Chromatography was performed with a Resource Q column. After several steps (see Section 2.1, protocol 1) retained proteins were eluted with a linear 12-min gradient from 20 m*M* Tris–HCl (pH 7.8), containing either 0.2% DDMAU or 0.2% DDMAB, to 0.5 *M* NaCl in the same buffer. The elution profile during the sodium chloride gradient is shown. The flow-rate was 5 ml/min for the wash steps and 1 ml/min for the sodium chloride gradient elution. The absorbance was monitored at 280 nm. Fractions of 2 ml were collected as indicated and analyzed by SDS–PAGE on 12.5% gels and by ELISA. In (c) the analysis of the fractions collected during chromatography (a) is shown. The SDS gel shown in (d) corresponds to the chromatogram of (b). The polypeptides were visualized by silver-staining. The arrows indicate the migration position of gD-1 and fragments thereof. E is the extract of the cells infected with the recombinant gD-1-baculovirus. The molecular masses ($\cdot 10^3$) of the reference proteins (R) are indicated. The concentration of gD-1 (μg/ml) in the fractions was determined by ELISA with the gD-specific mAb HD1 and they are indicated by black columns in the elution profile. (e) Immunoblot of fraction 5 of (c) obtained by using mAb A16 [21], peroxidase-conjugated rabbit antimouse IgG followed by diaminobenzidine staining. The molecular masses ($\cdot 10^3$) of reference proteins are indicated.

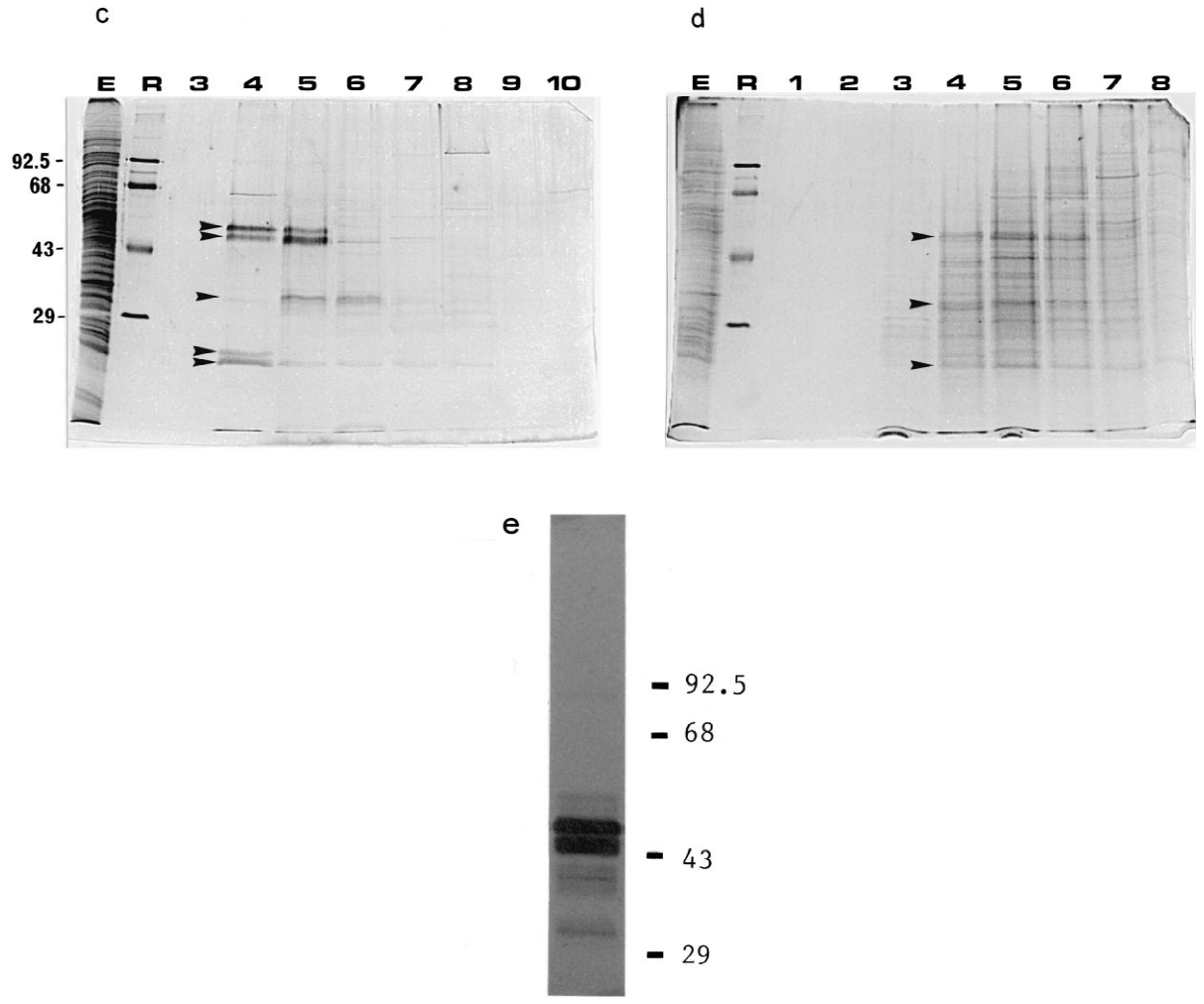

Fig. 1. (*continued*)

mAb (see Fig. 1a, black columns). The proteolytic degradation of gD-1 in extracts of insect cells is not unusual and has been described [22,27]. Chromatography in the presence of 0.2% DDMAB did not result in purification of gD-1 (Fig. 1d). Although some gD-1 was eluted (see Fig. 1b, black columns), it was eluted together with numerous other polypeptides. Chromatography in the presence of $C_{10}E_5$ (data not shown) showed results similar to those obtained in the presence of DDMAU. Chromatography of a $C_{10}E_5$ extract using a Mono Q column has been described earlier [15] and resulted in relatively pure conformationally intact gD-1. When the chromatography of a DDMAU and DDMAB detergent extract was performed using a Mono Q column with the same detergents as additives to the buffers, results were similar to using the Resource Q column, i.e., with DDMAU, virtually pure gD-1 was obtained, and with DDMAB no purification of gD-1 could be achieved.

The two detergents DDMAU and DDMAB have been used for the selective extraction [10] and purification [23] of membrane proteins of *Mycoplasma gallisepticum*. In these studies, the results show that extraction with DDMAU was relatively selective and that DDMAB had a higher efficiency in

membrane protein extraction. The addition of the detergent DDMAU (in a concentration of 2 m*M*, this is 16-times the CMC value) to the elution buffers for the HPIEC in that particular study resulted in purification of proteins p67, p52 and p77.

The above-mentioned results show that the strategy previously used for the purification of membrane proteins, using the detergent $C_{10}E_5$ [15–17], is also applicable when the detergent DDMAU was added to the elution buffers, but not when DDMAB was used as additive. It is easy to speculate that the difference in CMC of the detergents may account for these results. The elution buffers contained 0.2% detergent, which is 40-times the CMC of DDMAU, seven-times the CMC of $C_{10}E_5$, and 1.6-times the CMC of DDMAB. This of course does not exclude other possible factors like composition of the extract, and specific properties of the protein to be purified.

Since chromatographic results by following protocol 1, using either a Resource Q or Mono Q column were similar, a next set of experiments was performed, in which the gradient elution was performed at a flow-rate of 5 ml/min (protocol 2, HPIEC in Section 2.1). In Fig. 2, the results are shown of the chromatography of a DDMAU extract separated on a Resource Q column using a flow-rate of 5 ml/min. Glycoprotein D-1 is eluted relatively fast (see ELISA results, indicated as black columns in Fig. 2b) and mainly present in two fractions, fractions 2 and 3. The corresponding gel (Fig. 2a) shows that fractions 2 and 3 consist mainly of gD-1 and fragments thereof. Results obtained with the detergent $C_{10}E_5$ using the same protocol, are similar, gD-1, in almost

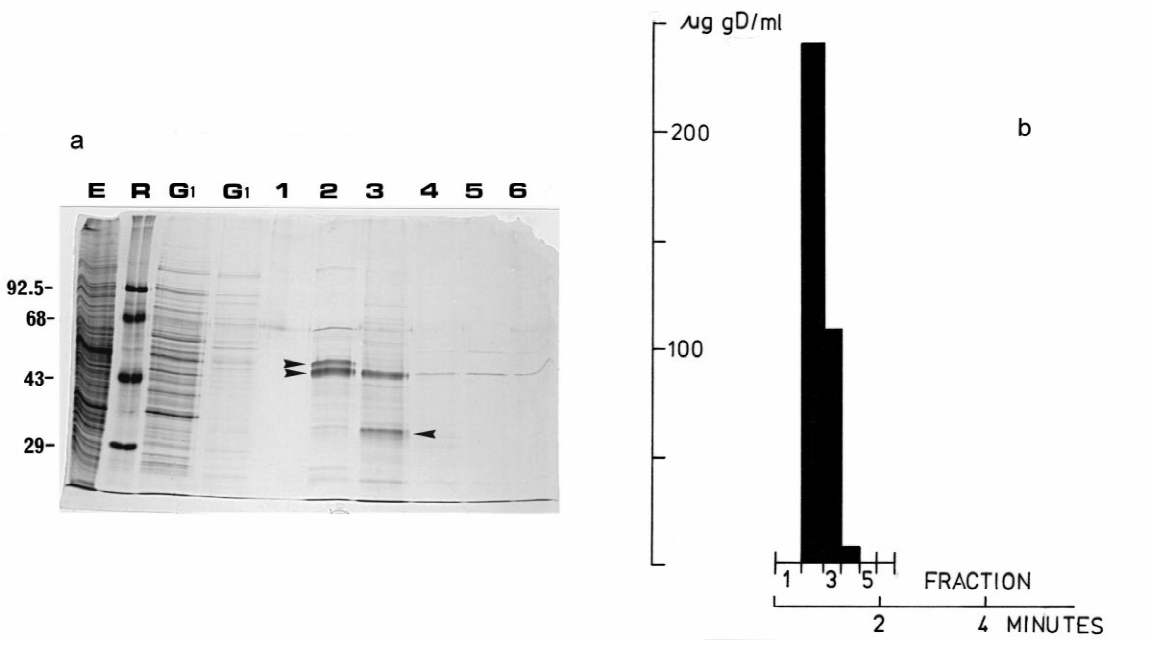

Fig. 2. HPIEC of a DDMAU extract of insect cells infected with a recombinant gD-1-baculovirus. Chromatography was performed with a Resource Q column. After several wash steps (see Section 2.1, protocol 2) retained proteins were eluted with a linear 12-min gradient from 20 m*M* Tris–HCl (pH 7.8), containing 0.2% DDMAU, to 0.5 *M* NaCl in the same buffer. The flow-rate was 5 ml/min for the wash steps and also for the sodium chloride gradient elution. The absorbance was monitored at 280 nm. Fractions of 2 ml were collected as indicated and analyzed by SDS–PAGE on 12.5% gels and by ELISA. In (a) the analysis of the fractions by SDS–PAGE is shown. The polypeptides were visualized by silver-staining. The arrows indicate the migration position of gD-1 and fragments thereof. E is the extract of the cells infected with the recombinant gD-1-baculovirus. G1 is the analysis of consecutive samples collected during the wash step with sodium chloride without detergent (step 2, protocol 2). Lanes 1–6 correspond to the fractions collected during the sodium chloride gradient with 0.2% DDMAU in the elution buffers. The molecular masses ($\cdot 10^3$) of the reference proteins (R) are indicated. The concentration of gD-1 (µg/ml) in the fractions was determined by ELISA with the gD-specific mAb HD1 and they are indicated by black columns in (b).

pure form, is eluted in two fractions (data not shown). Again, no purification at all was obtained using the detergent DDMAB as additive to the buffer.

Recoveries of gD-1 (Table 3) were determined by ELISA. As standard a serial dilution of a known concentration was included. The gD-1 concentration of the standard was determined by amino acid analysis [15]. The highest recovery of gD-1 was obtained either by chromatography of a $C_{10}E_5$ extract using a Mono Q column at a flow-rate of 1 ml/min or by chromatography of a DDMAU extract using a Resource Q column at a flow-rate of 5 ml/min.

4. Conclusions

The zwitterionic detergents DDMAU and DDMAB are equally effective in extracting the integral membrane protein gD-1 of herpes simplex virus from infected insect cells. In the purification of gD-1 from the detergent extracts by HPIEC, DDMAU (CMC 0.13 m*M*) was superior to DDMAB (CMC 4.3 m*M*) and similar to $C_{10}E_5$ (CMC 0.69 m*M*). This may suggest that detergents with a relatively low CMC are more useful as additive to elution buffers for HPIEC. The application of a Resource column, which allows a higher flow-rate than a Mono Q column reduced the chromatographic procedure from 64 min to 13 min.

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