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ALKALINE PHOSPHODIESTERASE I AND ALKALINE PHOSPHATASE I IN PLASMA MEMBRANES OF HERPES SIMPLEX VIRUS TYPE 1 TRANSFORMED HAMSTER CELLS

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Plasma membrane extracts from Herpes simplex virus type 1 transformed hamster embryo fibroblasts were chromatographed on *Lens culinaris* lectin coupled to Sepharose (LcH-Sepharose) and analysed by dodecyl sulphate polyacrylamide gel electrophoresis. Coomassie blue-staining revealed two major protein bands with apparent molecular weights of 125000 and of about 75000–90000. In plasma membranes isolated from these tumor cells prior labeled with [^{3}H]fucose or [^{3}H]glucosamine these bands contained the highest amounts of incorporated radioactivity. Separation by LcH-Sepharose-affinity chromatography as well as metabolic labeling clearly demonstrates their glycoprotein character. The 125000 protein coincides with alkaline phosphodiesterase I activity with a K_m of $6 \cdot 10^{-4}$ M for TMP *p*-nitrophenyl ester and is competitively inhibited by UDP-*N*-acetylglucosamine. This enzymatic activity is also present in normal hamster embryo fibroblasts. Gel electrophoresis of the *Lens culinaris* lectin-binding glycoproteins from plasma membranes of normal hamster embryo fibroblasts additionally revealed a strong alkaline phosphatase activity represented by an apparent molecular weight of 150000, while HSV_1 hamster tumor cells contain only a very weak activity of this enzyme activity. HSV-lytically infected cells, however, have unchanged levels of alkaline phosphatase activity, whereas alkaline phosphodiesterase activity increases slightly.

Introduction

In the literature data are accumulating that constituents of the cell surface membrane are involved in the molecular control of cellular processes such as growth, division and differentiation [1]. It has been suggested that membrane glycoproteins may serve as mediators of signals from the exterior to the interior of the cell which may result in these cellular processes [2]. The lack of detailed knowledge, however, on the relations between biochemically defined membrane glycoproteins and their membrane function is evident.

It is now well established that plasma membranes contain a considerable amount of enzymes and that dramatic changes of membrane associated activities occur after viral or chemical transformation of normal cells often accompanied by changes in the plasma-membrane glycoprotein pattern [1].

In this report we describe the isolation and characterization of two discrete glycoproteins from hamster embryo fibroblast plasma membranes and from Herpes simplex virus type 1 transformed hamster embryo fibroblasts propagated in newborn hamsters (denoted as HSV_1 hamster tumor cells).

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Abbreviation: HSV_1, Herpes simplex virus type 1.

Material and Methods

Cell culture. HSV_1 hamster tumor cells obtained as a kind gift from Dr. L. Thiry, Brussels, were injected subcutaneously at the back of 3 days old Syrian hamsters (10^6 cells in 0.1 ml phosphate buffered saline per animal). Tumors developed regularly in 100% of the inoculated animals within 2–3 weeks. After excision of the tumors, cell cultures could be easily established. The cells of these cultures were of a predominantly epithelial morphology with a minority of fibroblastic type cells. The cell cultures were routinely maintained in Eagle's basal medium with additional non-essential amino acids, 10% calf serum, 0.5% tryptose phosphate broth (Difco), streptothenate (200 μg/ml), penicillin (200 units/ml) and $NaHCO_3$ (6.0 mM) were propagated not further then 20 passages, and then new cultures were established from tumors induced by always the same HSV_1 hamster tumor stem line.

Primary rabbit kidney cells, BHK- and Vero-cells were grown in the same media. Herpes simplex viruses were grown and maintained as described earlier [22]. Infection was done with a multiplicity of infection of 5–10 per cell [22]. Lymphoid cell lines (Epstein Barr virus-transformed human B-lymphoid cells from Burkitt tumors, Raji-, Rael- and Akuba-cells) were raised and maintained as described earlier [33]. Virus strains Len, L3-2s, IES were of HSV type 1, strain D-316 of type 2 [22].

Plasma of plasma membranes. Plasma membranes were purified by the method of Kamat and Wallach [3] as modified for BHK 21 cells and described in detail by Gahmberg et al. [4]. The procedure is illustrated in Fig. 1.

Characterization of subcellular components by marker enzymes. Mg^{2+}-stimulated $(Na^+ + K^+)$-dependent ATPase (EC 3.6.1.3), alkaline phosphodiesterase I (EC 3.1.4.1) and alkaline phosphatase (EC 3.1.3.1) were used as plasma-membrane markers.

Possible contamination of the plasma-membrane fraction by mitochondria were checked using succinate dehydrogenase (EC 1.3.99.1). Lysosomal contamination was monitored by acid phosphatase (EC 3.1.3.2) activity and contaminating endoplasmic reticulum was checked by determination of NADPH diaphorase (EC 1.6.99.1) activity.

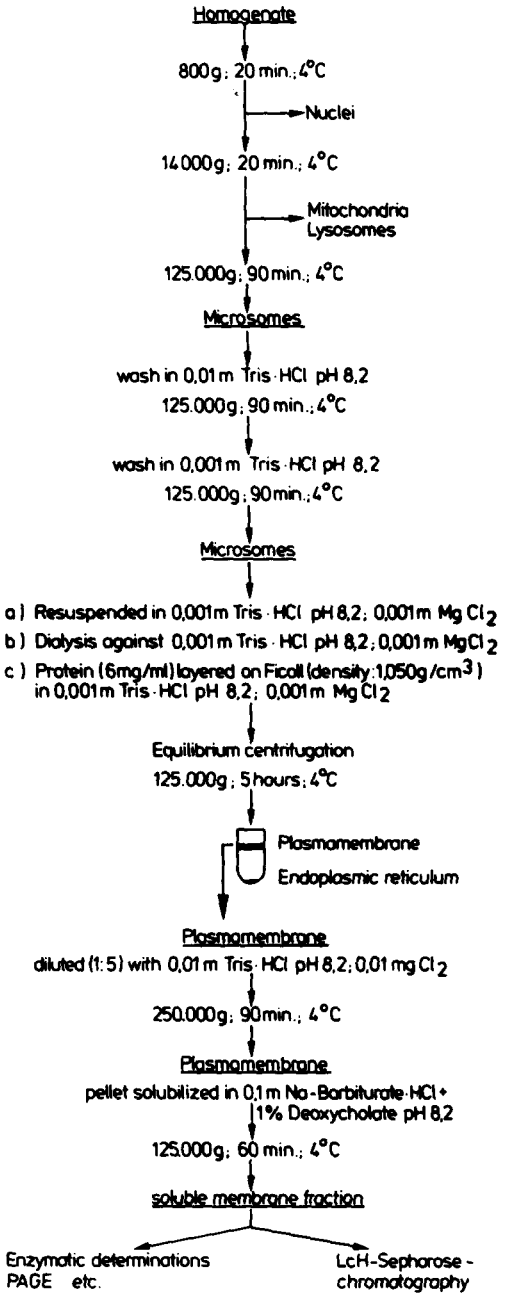

Fig. 1. Isolation of soluble plasmamembrane glycoproteins from HSV_1 hamster tumor cells for LcH-Sepharose chromatography.

Mg^{2+} stimulated $(Na^+ + K^+)$-dependent ATPase (EC 3.6.1.3) was assayed by the method of Schimmel et al. [5].

Alkaline phosphodiesterase I (EC 3.1.4.1) was

monitored spectrophotometrically by following the release of p-nitrophenol from thymidine-5'-monophosphate *p*-nitrophenyl ester [6]. Results were calculated by using $\epsilon_{405} = 1.20 \cdot 10^4$ [P · mol^{-1} · cm^{-1}].

Alkaline phosphatase I (EC 3.1.3.1) was assayed by the liberation of *p*-nitrophenol from *p*-nitrophenylphosphate in 2-amino-2-methylpropan-1-ol buffer (pH 9.8) at room temperature [7]. Otherwise gels were immersed into the same buffer, containing naphthol-AS-MX-phosphate. Under ultraviolet light (254 nm) the bright yellow-green fluorescence of naphthol-AS-MX was detectable when alkaline phosphatase was in the preparation. Then the gel was subjected to Coomassie blue-staining.

Acid phosphatase (EC 3.1.3.2) was checked with *p*-nitrophenyl phosphate as substrate according to Linhard and Walter [8].

Succinate dehydrogenase (EC 1.3.99.1) was assayed by the method of Sottocasa et al. [9].

Protein was determined by the method of Lowry et al. [10] with bovine serum albumin as standard.

Preparation of LcH-Sepharose 4B. The phytohemagglutinin from *Lens culinaris* seeds was isolated from commercial samples of lentils by a combination of the method described by Howard et al. [11] and Hayman et al. [12] (Rohrschneider, L., personal communication).

In brief, lentils were processed as described [11]. The protein fraction precipitated between 30% and 80% saturation of ammonium sulphate was added to Sephadex G-75, and the adsorbed *Lens culinaris* lectin eluted with 0.1 M α-methyl-D-glucopyranoside in 75 mM sodium phosphate buffer, pH 4.4 [12]. The buffers used in all these steps contained 10^{-4} M Mn^{2+} and Ca^{2+}. Coupling of *Lens culinaris* lectin (5.2 mg/ml) to cyanogen bromide activated Sepharose 4B (Pharmacia, Uppsala, Sweden) was performed in 0.1 M $NaHCO_3$, pH 8.4, containing 0.1 m α-methyl-D-glucopyranoside (free of Mn^{2+} and Ca^{2+}).

4 g of activated CNBr-Sepharose 4B were added to 10 ml of the lectin solution and rotated slowly end-over-end at 4°C overnight (about 20 h). The slurry was then mixed for 1 h in 5 mM ethanolamine-HCl (pH 8.4) to cover unreacted CNBr sites on the Sepharose and washed successively with 0.1 M $NaHCO_3$, pH 8.4, water, 0.1 M acetate buffer, pH 6.0, containing 10^{-4} M Ca^{2+} and 10^{-4} M Mn^{2+}. LcH-Sepharose was stored in 0.1 M acetate buffer (pH 6.0) 10^{-4} M Ca^{2+}, 10^{-4} M Mn^{2+} at 2°C.

Chromatography of plasma membrane on LcH-Sepharose. Chromatography of sodium deoxycholate solubilized plasma-membrane material was performed in a system similar to that described by Snary et al. [13]. Throughout the experiments 20 ml of LcH-Sepharose containing columns (1 × 15 cm) were used. Equilibration of the columns was achieved by washing with 10 vols. of each of the following buffers at room temperature: 0.01 M acetate buffer (pH 6.8), 0.1 m barbiturate-HCl buffer (pH 8.2), 1% deoxycholate in 0.1 M barbiturate-HCl buffer (pH 8.2), containing 0.2 M α-methyl-D-mannopyranoside and finally 1% deoxycholate in 0.1 M barbiturate-HCl buffer (pH 8.2). All solutions contained 0.02% sodium azide. The deoxycholate-solubilized plasma membrane material (50 mg) was passed through the column and then equilibrated for 2 h after the first slight increase of adsorbance at 280 nm was to be seen. Then the column was washed with 1% deoxycholate in 0.1 M barbiturate-HCl buffer (pH 8.2). The specifically bound material was eluted with 1% deoxycholate in 0.1 M barbiturate-HCl buffer (pH 8.2) containing 0.2 M α-methyl-D-mannopyranoside and eluate was concentrated by ultrafiltration. (We omitted Mn^{2+} ions in the sample and elution buffers to avoid the precipitation of manganese oxide which appears at pH > 7 and influences the elution characteristics of the LcH-Sepharose column.)

Gel electrophoresis. Discontinuous polyacrylamide gel electrophoresis in sodium dodecyl sulphate (1.0%) was performed in a system according to Davis [14] and as described in detail by Maurer [15] with a 3.5% stacking gel and a 7.5% separation gel in 0.025 M Tris-0.19 M glycine buffer (pH 8.9). Radioactively labeled proteins were analysed using cylindrical gels (10 cm × 0.6 cm). Immediately after electrophoresis these gels were sliced into 2 mm fractions (Gilson gel fractionator; Gilson, Valliers-Le Bel, France), mashed and deposited into scintillation vials. For solubilization 0.5 ml of H_2O_2 (30%) were added to each vial further treated at 60°C for 12 h. After addi-

tion of 10 ml of Bray's solution [16] to each sample radioactivity was measured in a Packard Tricarb Scintillation spectrometer (Model 3380).

To compare protein patterns and to determine the positions of alkaline phosphodiesterase and alkaline phosphatase, respectively, electrophoresis was performed on 7.5% polyacrylamide slab gels ($10 \times 10 \times 0.25$ cm) containing only 0.1% sodium dodecyl sulphate. After electrophoresis gels were treated with a solution of Coomassie blue [17] to stain protein bands. Molecular weights of the proteins were determined by the method of Weber and Osborne [18].

Specific staining procedures for detection of enzymes. To determine the position of the alkaline phosphodiesterase activity immediately after electrophoresis the gel was immersed in 5 mM TMP *p*-nitrophenyl ester (dissolved in 0.1 M Tris-HCl, pH 8.9).

The position of the enzyme indicated by the developing yellow band was marked by razor blade cuts. Additionally, gels were cut with a Gilson gel fractionator and the enzyme activity was determined directly in the fractions. In a second step the slab gels were stained with Coomassie blue to determine the coincidence of enzyme position and protein bands.

The position of alkaline phosphatase activity was determined by the method of Trepanier et al. [19]. For this purpose, the slab gel was immersed in 1.8 M 2-amino-2-methylpropan-1-ol-HCl buffer (pH 9.8) containing 6 mM Naphthol-AS-MX-phosphate (Sigma, St. Louis, MO, U.S.A.), 6 mM $MgCl_2$, 10^{-4} M $ZnCl_2$ and 0.1% sucrose (w/w). The development of a green-yellow band visible under ultraviolet light indicated the position of the alkaline phosphatase. Its position was also marked by razor blade cuts, and then the gel was stained with Coomassie blue. Furthermore, stained gels were scanned in a Chromoscan-apparatus (Joyce-Loebl) and the absorption was registered continuously.

Results

Isolation of plasma membranes from HSV_1 hamster tumor cells involved the separation of a microsomal fraction from cells disrupted by microcavitation in a nitrogen pressure homogenizer (Artisan, Waltham, MA, U.S.A.) by sedimentation through a barrier of Ficoll [3]. In the presence of divalent cations plasma membranes remained at the buffer-ficoll interphase, whereas most of the smooth internal membranes and microsomal components pelleted through the ficoll barrier [3]. Purification of the plasma-membrane fraction was followed by testing the activity of enzymes which are known as markers for the different subcellular particles. The values shown in Table I are in good agreement with data obtained by other investigators [4] for BHK cells, and are indicative of a high degree of enrichment of plasma membrane material. From the table also the accumulation of [^{3}H]fucose in the plasma-membrane fraction can be seen after metabolic labeling of the cells.

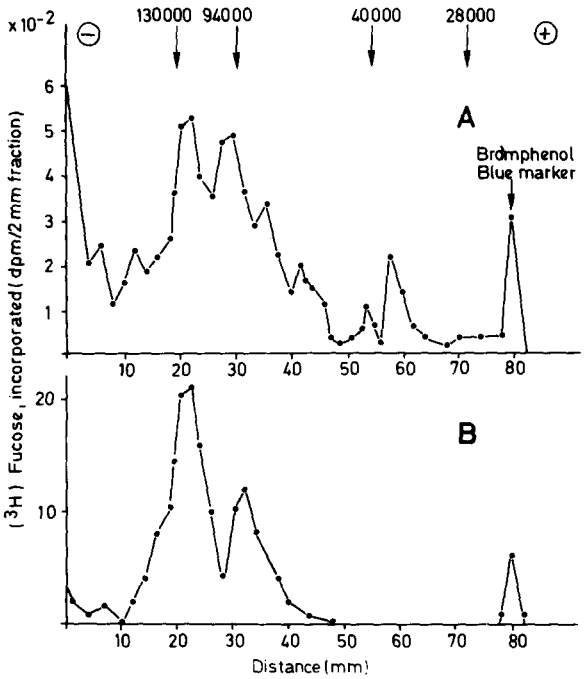

Fig. 2. Analysis of [^{3}H]fucose labeled plasma-membrane glycoproteins isolated from HSV_1 hamster tumor cells on cylindrical sodium dodecyl sulphate polyacrylamide gels. Samples from plasma membranes (A) and plasma-membrane glycoproteins bound to LcH-Sepharose and specifically eluted with α-methyl-D-mannopyranoside (B) were subjected to sodium dodecyl sulphate polyacrylamide gel electrophoresis in cylindrical gels and the gels processed for determination of radioactivity as described in Materials and Methods. As marker proteins β-galactosidase, phosphorylase *a*, aldolase and concanavalin A were used displaying apparent molecular weights of 130000, 94000, 40000 and 28000, respectively [18,36].

Table I

DISTRIBUTION OF MARKER ENZYMES DURING THE STEPS OF PREPARATION OF THE PLASMA-MEMBRANE GLYCOPROTEIN FRACTION BINDING TO LcH-SEPHAROSE

Results are averages from three fractionations: spec. act. range was ±10% α MM, α-methyl-D-mannopyranoside; SA, specific activity; Enr., enrichment factor.

Fraction	Protein (mg)	$(Na^+ + K^+)$-activated ATPase		Alkaline Phospho-diesterase		Alkaline Phosphatase		Acid Phosphatase		Succinate dehydro-genase		[^{3}H]Fucose (dpm/mg protein)	NADPH diaphorase	
		SA [a]	Enr.	SA [b]	Enr.	SA [a]	Enr.	SA [d]	Enr.	SA [e]	Enr.		SA [f]	Enr.
Homogenate	3500 (100%)	7.8	1	6.	19	0.3	1	29.1	1	9.3	1	$2 \cdot 10^3$	36.2	1
Microsomes	550 (15.7%)	32.3	4.1	28.1	4.1	0.28	–	1.2	0.05	0		$6.7 \cdot 10^3$	0.05	
Plasma membrane	90 (2.6%)	96.3	12.4	170.8	24.7	0.3	–	0	0	0		$15 \cdot 10^3$	–	
LcH-Sepharose α MM-Fraction	7 (0.2%)	n.d.		550	79.7	0.3	–	n.d.		n.d.		$52 \cdot 10^3$	n.d.	

[a] nmol inorganic phosphate liberated per mg protein per min.
[b] [c] and [d] nmol *p*-nitrophenolate liberated per mg protein per min.
[e] nmol cytochrome *c* reduced per mg protein per min.
[f] nmol NADPH oxidized per mg protein per min.

[^{3}H]fucose in the plasma-membrane fraction can be seen after metabolic labeling of the cells.

The plasma membranes collected at the ficoll-buffer-interphase by high speed centrifugation (Spinco, SW40 rotor, 125000 × *g*; 60 min; 4°C) were dissolved in 0.1 M barbiturate buffer (pH 8.2) containing 1% sodium deoxycholate similar to the originally described procedure by Allen et al. [20] for pig lymphocyte plasma membranes. About 80% of the protein and 90–95% of the [^{3}H]fucose label of the plasma-membrane pellet was found in the clear supernatant after high centrifugation (Spinco, SW40 rotor; 200000 × *g*; 50 min; 4°C). This supernatant was then used for affinity chromatography on LcH-Sepharose.

To distinguish glycosylated from nonglycosylated protein components of the plasma membrane, metabolic labeling of the cell surface components with either [^{3}H]fucose or [^{3}H]glucosamine was performed since both glucosamine and fucose are precursors incorporated specifically into glycoproteins [21]. For comparison, both labeled plasma-membrane extracts from HSV_1 hamster tumor cells and plasma-membrane glycoproteins of these cells isolated by LcH-Sepharose affinity chromatography were subjected to SDS polyacrylamide gel electrophoresis in cylindrical gels. The obtained radioactivity profiles are shown in Fig. 2.

The separation of plasma-membrane glycoproteins of HSV_1 transformed tumor cells (Fig. 2A) revealed four major radioactive peaks with apparent molecular weights of about 130000, 95000, 60000 and 35000. As concluded from Fig. 2B chromatography of the plasma-membrane fraction on LcH-Sepharose revealed two peaks with apparent molecular weights of 125000 and 95000 indicating that two out of the four major plasma-membrane glycoproteins contain glycoproteins with α-D-mannopyranosyl- or glucopyranosyl-containing carbohydrate chains. Specific binding to the *Lens culinaris* lectin results in separation of these glycoproteins from the bulk of the plasma-membrane proteins and glycoproteins.

Alkaline phosphodiesterase and alkaline phosphatase activity of membrane glycoproteins

Initial experiments were designed to determine the relative sodium dodecyl sulphate-stability of these enzymes. In Table II is shown that sodium dodecyl sulphate present in the incubation mixture up to 1.5% seems to enhance the activity of alkaline phosphodiesterase slightly. After infection this activity was found somewhat higher.

TABLE II

ACTIVITY OF ALKALINE PHOSPHODIESTERASE FROM BHK-CELLS IN THE PRESENCE OF SODIUM DODECYL SULPHATE

SDS concn. (%)	Noninfected extract tested		HVS-infected extract tested	
	Directly	After preincubation [a]	Directly	After preincubation [a]
–	40.0 [b]	80.0	64.0	134.0
0.05	30.0	70.0	74.0	162.0
0.1	72.0	124.0	78.0	158.0
0.75	86.0	120.0	128.0	190.0
1.5	156.0	200.0	166.0	222.0

[a] The extract was preincubated for 180 min in the presence of varying concentrations of sodium dodecyl sulphate.
[b] Activity expressed in nmol/mg protein per 60 min.

In order to detect the protein band which corresponds to alkaline phosphodiesterase activity, slab gel electrophoresis was used including sodium deoxycholate (1%) and sodium dodecyl sulphate (0.1%) in the separation gel.

To obtain strong reactions in relative short staining periods (15–30 min) we had to use samples of about 250–300 μg protein per sample well of the slab gel. This high protein concentration and possibly diffusion during the staining procedure could explain the broadening of the bands compared to those from Coomassie blue staining.

After immersion of the slab gel in a solution of TMP *p*-nitrophenyl ester a yellow band indicated the position of the alkaline phosphodiesterase activity. After marking the position of the yellow band the gel was subjected to Coomassie blue staining and the pattern of Fig. 3, column a, was obtained. In parallel, such gels were cut into slices and the enzymatic activity was determined (Fig. 3). Enzyme activity and Coomassie blue band with apparent molecular weight of 125000 coincide well.

In order to correlate more exactly alkaline phosphodiesterase activity to the Coomassie blue

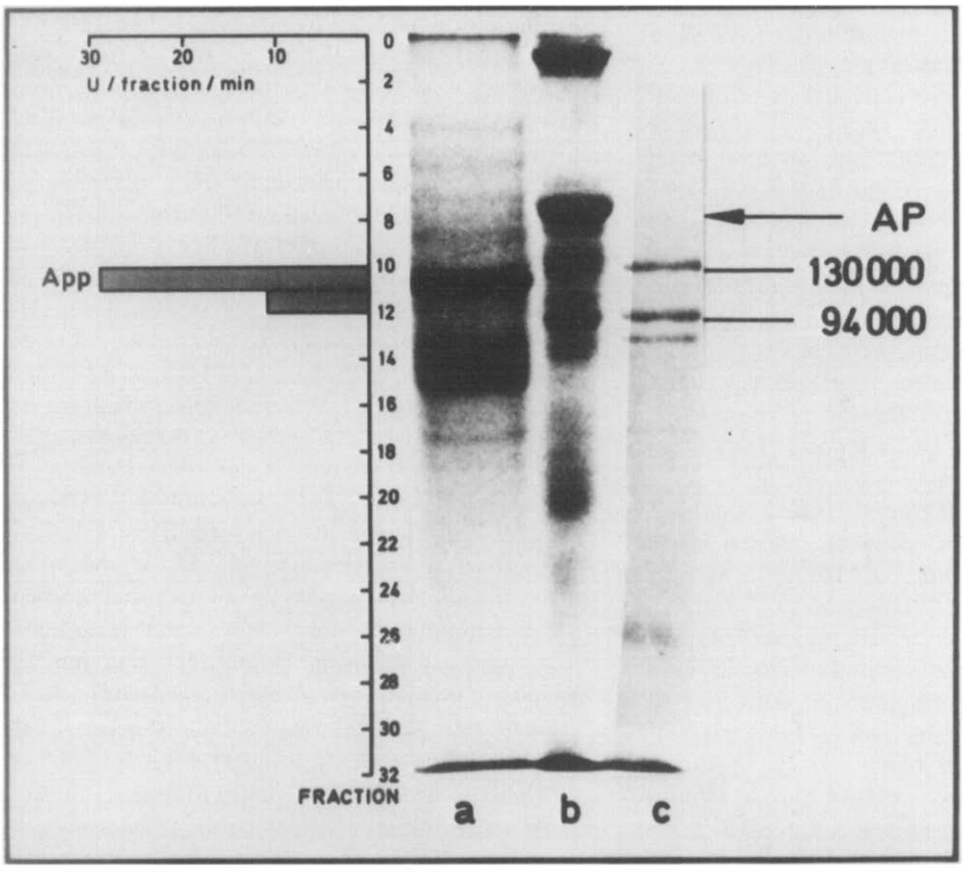

Fig. 3. Electrophoretic comparison of *Lens culinaris* lectin-binding glycoproteins from plasma membranes of HSV_1 hamster tumor cells (a) and primary hamster embryo fibroblasts (b). Samples (250 μg per slot) were prepared from both plasma membranes by chromatography on LcH-Sepharose and subsequent elution of the bound glycoproteins with α-methyl-D-mannopyranoside, electrophoresis in sodium dodecyl sulphate polyacrylamide slab gels and stained for enzyme and protein localization as described under Materials and Methods. App, alkaline phosphodiesterase I activity; AP, alkaline phosphatidase I activity. Column c shows the position of marker proteins β-galactosidase and phosphorylase *a* which display apparent molecular weights of 130000 and 94000.

stained gels, we analysed the position of both the yellow enzyme activity band and the Coomassie blue band by scanning the gels. 80 μg of lectin purified non-infected cell material was used per gel. Coomassie blue staining revealed two main bands with apparent molecular weights of 125000 and 75000–90000.

As shown in Fig. 4a + b only the band with apparent molecular weights of 125000 from Coomassie blue staining corresponds to the enzyme activity.

Experiments to detect alkaline phosphatase activity were performed similarly since alkaline phosphatase activity is not destroyed in solutions containing up to 1% dodecyl sulphate [23]. In the case of plasma-membrane extracts from primary hamster embryo cells bound to and eluted from LcH-Sepharose (Fig. 3, column b) three main bands with apparent molecular weights of 150000 (designated alkaline phosphatase), 130000 and 90000 were found. The 150000 band was identified as alkaline phosphatase, the 130000 band as alkaline phosphodiesterase.

We conclude that the 125000 band in lane a (Fig. 3) corresponds to the 130000 band of lane b and that in both cases the stained glycoprotein corresponds to alkaline phosphodiesterase. The difference in apparent molecular weight could be

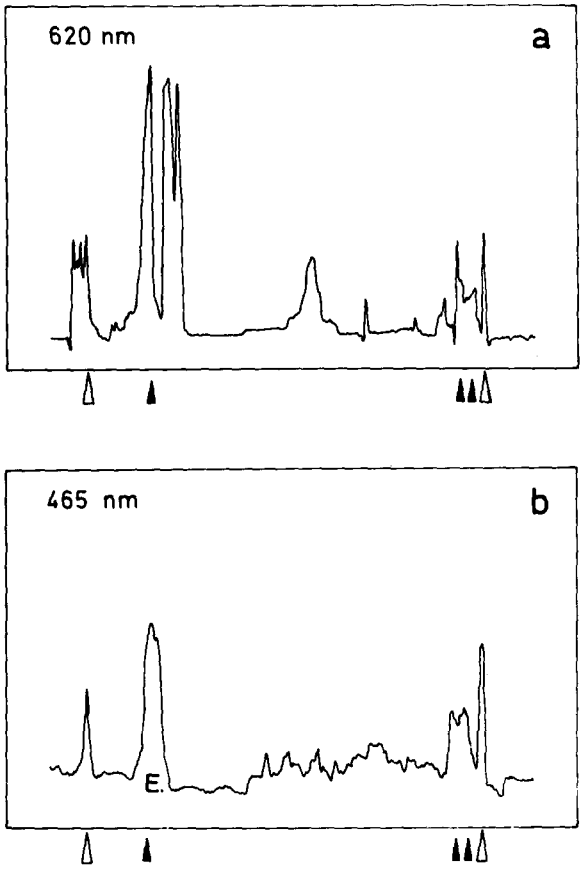

Fig. 4. Coomassie blue (a) and alkaline phosphodiesterase staining (b) of *Lens culinaris* lectin-purified extracts of HSV-transformed hamster tumor cells after polyacrylamide gel electrophoresis (12% gel). The Coomassie blue-stained gel was scanned at 620 nm, and the *p*-nitrophenolate-stained gel was scanned at 465 nm.

the consequence at different processing by the two cell types.

Furthermore, Fig. 3 clearly demonstrates that in the *Lens culinaris* lectin-binding glycoprotein fraction of plasma membranes from HSV_1 hamster tumor cells the 150000 band is absent (lane a) while present in the *Lens culinaris* lectin-binding glycoprotein fraction of plasma membranes from untransformed hamstre cells (lane b). The absence of this band is in agreement with the lack of alkaline phosphatase activity in the plasma-membrane preparation as can be seen from Table I.

Enzymatic properties of alkaline phosphodiesterase

Evans et al. [24] have shown that alkaline phosphodiesterase isolated from mouse liver plasma membranes consists of a single polypeptide chain possessing dual specificity towards phosphodiester and nucleotide pyrophosphate substrates such as TMP *p*-nitrophenyl ester, ATP, NAD^+, UDP-Gal and UDP-*N*-acetylglucosamine.

We therefore studied the influence of UDP-*N*-acetylglucosamine on the alkaline phosphodiesterase activity when TMP *p*-nitrophenyl ester was used as substrate. For this purpose the LcH-Sepharose binding glycoproteins were eluted from gel slices with water and used for Michaelis-Menten kinetic studies. The K_m was determined to be $6.0 \cdot 10^{-4}$ M. As can be seen from the Lineweaver-Burk plots in Fig. 4, the activity of alkaline phosphodiesterase (with TMP *p*-nitrophenyl ester as substrate) is competitively inhibited by UDP-*N*-acetylglucosamine with a K_i of $1.4 \cdot 10^{-4}$ M.

From this result it is tempting to conclude that the alkaline phosphodiesterase isolated from hamster tumor cell plasma membranes also has alkaline nucleotide pyrophosphatase activity as is known for the alkaline phosphodiesterase from plasma membranes of rat liver [25] and mouse liver [24], respectively.

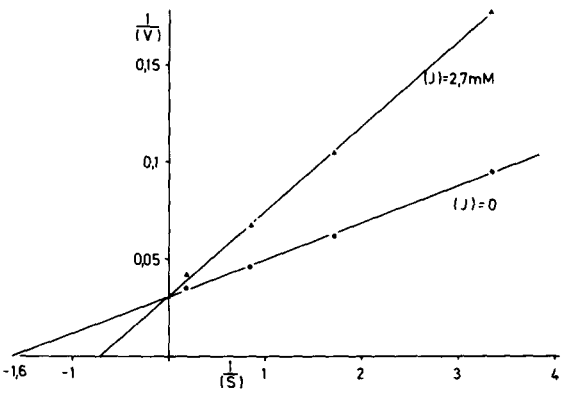

Fig. 5. Competitive inhibition of alkaline phosphodiesterase I from HSV_1 hamster tumor cell plasma membranes with TMP *p*-nitrophenyl ester as substrate by UDP-*N*-acetylglucosamine. Alkaline phosphodiesterase I was extracted from polyacrylamide slab gels as described under Materials and Methods and subjected to Michaelis-Menten kinetic studies. *S*, mM TMP *p*-nitrophenyl ester; *V*, mM *p*-nitrophenolate/min per mg protein (25°C); *J*, mM UDP-*N*-acetylglucosamine.

TABLE III

THE ACTIVITY OF ALKALINE PHOSPHATASE AND ALKALINE PHOSPHODIESTERASE IN LYMPHOID CELLS AND IN HSV-INFECTED PRIMARY RABBIT KIDNEY CELLS

Cell type	High multiplicity of infection with HSV-strains	Alkaline phosphatase [a]	Alkaline phosphodiesterase [a]
Raji	–	1.8	n.d.
Rael	–	9.6	n.d.
Akuba	–	1143.3	95
Primary rabbit kidney cell	–	127	22
	Len	138	47
	L3-2s	152	74
	IES	126	16
	D-316	175	69
BHK	–	1.6	163.8
Vero	–	201	244.8

[a] nmol released per mg protein per 60 min.

Alkaline phosphatase and alkaline phosphodiesterase activity in different cell lines and after infection with HSV

To confirm the results concerning the lack of alkaline phosphatase activity in HSV transformed cells some lymphoid cell lines, BHK-, Vero- and primary rabbit kidney cells were tested for alkaline phosphatase and alkaline phosphodiesterase activity. Primary rabbit kidney cells were also tested after infection with some strains of HSV.

Table III shows that in BHK-, Raji- and Rael-cells alkaline phosphatase activity is very low. Akuba-, Vero- and primary rabbit kidney cells in contrast demonstrate high activities. Infection of primary rabbit kidney cells with HSV-strains does not influence the activity of the alkaline phosphatase. After infection (except HSV-1 strain IES) of primary rabbit kidney cells the álkaline phosphodiesterase activity increases slightly. This can also be seen in BHK cells (Fig. 4).

Discussion

Approx. 10% of the sodium deoxycholate solubilized plasma membrane components of HSV_1 hamster tumor cells were specifically bound to LcH-Sepharose. Sodium dodecyl sulphate polyacrylamide gel electrophoretic analysis of these lectin binding glycoproteins revealed two major Coomassie blue-staining bands, one with apparent molecular weights of 125000 and another broad one with about 75000–90000. This latter band possibly contains more than one glycoprotein species.

These results were confirmed by experiments with HSV_1 hamster tumor cells grown in the presence of [^{3}H]fucose and [^{3}H]glucosamine, respectively, which revealed two major bands with apparent molecular weights of 125000–130000 and 95000, characterized by high incorporation of [^{3}H]fucose or [^{3}H]glucosamine.

In comparison, plasma membranes of primary hamster fibroblasts investigated by the same procedures also contained the *Lens culinaris* lectin-binding glycoprotein with the apparent molecular weight of 130000 and identical data were obtained from plasma membranes of HEp-2- and BHK-cells (data not shown).

As reported by other authors the observed glycoprotein with apparent molecular weight of 130000 displays specificity for the hemagglutinins of *Lens culinaris* and *Ricinus communis* in normal and transformed cells [26–28].

Several authors have demonstrated alkaline phosphodiesterases as constituent of plasma membranes (see Refs. in Ref. 27) and Evans et al. [24] have shown that plasma membranes isolated from mouse liver contained an alkaline phosphodiesterase activity which gave a single glycoprotein band in the polyacrylamide gel with apparent molecular weight of 128000–130000. Our results show that this enzymatic activity is enriched in the plasma-membrane fraction which degrades TMP *p*-nitrophenyl ester and that this activity relatively increased in the glycoprotein fraction specifically bound to and eluted from LcH-Sepharose with α-methyl-D-mannopyranoside.

Staining of the polyacrylamide gels used for separating the two main components of the LcH-Sepharose fraction with TMP *p*-nitrophenyl ester demonstrated that the glycoprotein with apparent molecular weight of 125000 is an alkaline phosphodiesterase. It's K_m was determined to be $6.0 \cdot 10^{-4}$ M.

From the work of other investigators [24,25] there is evidence that the alkaline phosphodiesterase from mouse and rat liver plasma membranes are active against both phosphodiester and nucleotide pyrophosphate bonds and have to be considered generally as alkaline nucleotide pyrophosphatases. We found that the phosphodiesterase activity was competitively inhibited by UDP-β-acetylglucosamine and the K_i was determined to be $1.4 \cdot 10^{-4}$ M. It is tempting to conclude that the glycoprotein with apparent molecular weight of 125000 from HSV_1 hamster tumor cell plasma membranes also has the properties of an alkaline nuleotide pyrophosphatase.

During the characterization of our plasma-membrane preparations from HSV_1 hamster tumor cells by means of enzymatic markers we were puzzled by the fact that the enzymatic activities of 5'-nucleotidase and alkaline phosphatase widely used as plasma-membrane marker were very low or absent in our preparations and that no alkaline phosphatase activity was detectable compared to the high activity of this enzyme in primary hamster embryo fibroblasts. Sodium dodecyl sulphate gel analysis showed that the *Lens culinaris* lectin-binding glycoprotein fraction from plasma membranes of primary hamster embryo fibroblasts contained a glycoprotein with apparent molecular weight of 150000 showing alkaline phosphatase activity. In contrast, the appropriate fraction from HSV_1 hamster tumor and BHK cells showed no alkaline phosphatase activity.

This activity, however, has been shown to be inducible by BdU or by BdU and prednisolon [35]. In an earlier study Sela et al. [30] reported that hamster cell lines transformed by *Polyoma* virus or by treatment with dimethylnitrosamine were blocked in their ability to hydrolyze *N*-acetyl-D-hexosamine 1-phosphate to *N*-acetyl-D-hexosamine [29]. They have shown that the blocked phosphomonoesterase was an alkaline phosphatase [30]. Recently Bader et al. [31] reported that chick embryo cells transformed by two different strains of *Rous sarcoma* virus and rat embryo cells transformed by murine sarcoma virus had lower levels of alkaline phosphatase activity compared to non-transformed chick embryo cells and rat embryo cells, respectively.

Our results demonstrate that in HSV_1 hamster tumor cells the activity of alkaline phosphatase is strongly reduced compared to untransformed cells, and the corresponding protein band is not detectable by polyacrylamide gel electrophoresis. In human B-lymphoid lines the alkaline phosphatase activity varies considerably (Table III) whereas in mouse B-cell lines the activity is generally very high [32]. The loss does not seem to be an invariable marker for tumor cells [34]. No alterations of alkaline phosphatase activity however could be observed in lytically Herpes simplex virus-infected primary rabbit kidney cells. After infection, the activity of the alkaline phosphodiesterase increases slightly in BHK-cells.

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