Journal of Supramolecular Structure 12:115–125 (1979)
Tumor Cell Surfaces and Malignancy 481–491

Ehrlich Ascites Tumor Cell Surface Labeling and Kinetics of Glycocalyx Release

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Ehrlich ascites tumor cells spontaneously release cell surface material (glycocalyx) into isotonic saline medium. Exposure of these cells to tritium-labeled 4,4′-diisothiocyano-1,2-diphenylethane-2,2′-disulfonic acid ($^3H_2$DIDS) at 4°C leads to preferential labeling of the cell surface coat. We have combined studies of the kinetics of $^3H_2$DIDS-label release, the effects of enzymatic treatment, and cell electrophoretic mobility to characterize the $^3H_2$DIDS-labeled components of the cell surface. Approximately 73% of the cell-associated radioactivity is spontaneously released from the cells after 5 h at 23°C. The kinetics of release is consistent with the first-order loss of two fractions; a slow ($\tau_{1/2}$ = 360 min) component representing 33% of the radioactivity, and a fast ($\tau_{1/2}$ = 20 min) component representing 26%. The remaining 14% of the labile binding may reflect mechanically induced surface release. Trypsin (1 μg/ml) also removes approximately 73% of the labeled material within 30 min and converts the kinetics of release to that of a single component ($\tau_{1/2}$ = 5.5 min). The specific activity (SA) of material released by trypsin immediately after labeling is 83% of the SA of the material spontaneously lost in 1 h. However, trypsinization following a 2-h period of spontaneous release yields material of reduced (43%) SA. Neither $^3H_2$DIDS labeling nor the initial spontaneous loss of labeled material alters cell electrophoretic mobility. However, extended spontaneous release is accompanied by a significant decrease in surface charge density. Trypsinization immediately following labeling or after spontaneous release (2 h) reduces mobility by 32%. We have tentatively identified the slowly released compartment as contributing to cell surface negativity.

Key words: glycocalyx, cell surface, tumor cells, Ehrlich ascites tumor, surface labeling

INTRODUCTION

The external cell surface coat or glycocalyx [1] of animal cells represents a biochemically and functionally separate fraction of the cell membrane. It has been implicated as playing a major role in cell adhesion, cell recognition, and the establishment or modulation of antigenic properties of the cell [2, 3]. Perhaps related to these functions, the oligosaccharide portions of the glycocalyx impart a net surface negative charge to the cell [4].

Comparison of the peripheries from normal and malignant cells have demonstrated differences in the functional activities of glycoproteins [5], glycolipids [6], and surface

Received March 18, 1979; accepted August 17, 1979.

0091-7419/79/1201-0115$02.30

antigens [3, 7]. Alterations in the oligosaccharide composition of cell glycoproteins have also been noted upon transformation of BHK cells [8], leading to the hypothesis that changes in the bound carbohydrates signal malignant behavior. Loss of the glycocalyx may also be involved in tumorigenicity. The glycocalyx is a labile structure which can be removed from a variety of cells, including tumor cells, by mild treatments such as repetitive washing [9]. Malignant cells, in particular, shed glycoproteins into their environment [10]. Consequently, spontaneous shedding of surface antigens may provide a mechanism for avoidance of immunologic destruction [11].

Ehrlich ascites tumor cells have been shown to spontaneously release a variety of surface iodinated glycoproteins and glycosaminoglycans from lactoperoxidase-labeled cells after incubation in physiologic salt solution for 1 h at 4°C [12]. Comparison of enzymatic activity, and protein and sialic acid contents of the glycocalyx, with those of the plasma membrane demonstrated a clear distinction between these cellular fractions.

Recently, we have utilized the transport inhibitor, H_2DIDS (4′,4′-diisothiocyano-1,2-diphenylethane-2,2′-disulfonic acid), to investigate anion transport in Ehrlich ascites tumor cells [13, 14]. Incubation of cells in the presence of H_2DIDS at 21°C results in an irreversible binding of the agent to components of the cell surface. As a consequence of binding, inhibition of sulfate transport occurs. However, cells incubated with H_2DIDS at low temperature (1–5°C) irreversibly bind the agent without concomitant transport inhibition.

Furthermore, cells labeled at either low temperature or 21°C spontaneously release H_2DIDS-labeled surface proteins into their environment. In the case of transport-inhibited cells, this spontaneous release does not relieve inhibition. Protein collected from the medium is significantly enriched with respect to H_2DIDS-binding compared to the whole-cell protein under all labeling conditions. These results suggest that H_2DIDS may be used as a marker for cell surface material under mild conditions which do not compromise cell integrity or viability.

While it is clear that cell surface components of these cells are labile, no studies concerning the rate of loss, or indeed whether all components are equally labile, have been reported. In the present investigation, we have employed tritiated H_2DIDS ($^3H_2$DIDS) as a label for cell surface material. We have combined studies of the kinetics of release of $^3H_2$DIDS-labeled surface components, and the effects of trypsin, on this process to characterize cell surface dynamics. In addition, the effect of glycocalyx removal on cell electrophoretic mobility was determined.

MATERIALS AND METHODS

Cell Suspension

Experiments were performed with Ehrlich-Lettré ascites tumor cells (hyperdiploid strain) which were maintained in Ha/ICR male mice by weekly transplantations. Tumor-bearing animals with growths of 8–11 days were used. Cells were removed from unanesthetized animals by peritoneal aspiration and washed free of ascitic fluid by gentle centrifugation and resuspension. The wash and resuspension solution had the following composition: 135 mM NaCl, 10 mM Na_2SO_4, 7 mM KCl, and 10 mM Hepes-NaOH (pH 7.2–7.3; 296–301 mOsm). All experiments were also carried out in this medium. Cell suspensions (20–40 ml; 100 mg wet mass per milliliter) were placed in Ehrlenmeyer flasks under air atmosphere and incubated in an ice-bath for 5 min prior to use.

Reagents

Tritium-labeled 4,4′-diisothiocyano-1,2-diphenylethane-2,2′-disulfonic acid ($^3H_2$DIDS) was synthesized as previously described [14]. Trypsin (twice crystallized), trypsin inhibitor (from soybean), and neuraminidase (from Cl. perfringens) were products of Sigma Chemical Co.

$^3H_2$DIDS Labeling of the Cells

Cells, maintained in the resuspension medium, were incubated in the presence of $^3H_2$DIDS (25 μM) for 30 min at 1–4°C. The interaction between $^3H_2$DIDS and the cells was stopped by the addition of ice-cold medium containing 0.5% bovine serum albumin (Sigma Chemical Co.). Usually 40 ml of cold albumin wash solution was added per 10 ml cell suspension. The cells were separated from the medium by centrifugation (1,500g for 1 min) and the packed cells were then washed once in ice-cold albumin solution and twice in ice-cold, albumin-free medium. The labeled, packed cells were resuspended at 23°C. This procedure results in an interaction of $^3H_2$DIDS with the tumor cell which is restricted to the cell surface and membrane, but which does not lead to inhibition of anion transport [14]. Aliquots of the labeled cell suspension were removed for the determination of wet and dry weight [15] and total $^3H_2$DIDS binding [14] as previously described.

Kinetics of $^3H_2$DIDS-Labeled Glycocalyx Release

To assess the spontaneous release of labeled glycocalyx, aliquots (0.2 ml) of the labeled cell suspension were removed periodically (1–120 min) and centrifuged (15,000g; 1 min) and the supernatant was assayed for radioactivity.

The effect of trypsin (1 μg/ml) on glycocalyx release was investigated in two ways. First, cells were permitted to spontaneously release glycocalyx for 2 h; then they were centrifuged (ca. 1,600g; 1 min) and resuspended in fresh medium containing trypsin. Aliquots (0.2 ml) were removed periodically (1–30 min) and the supernatant was assayed for radioactivity. In other studies, freshly labeled, washed cells were resuspended directly into medium containing trypsin (1 μg/ml). Loss of glycocalyx was followed for 30 min as described above.

Aliquots of cell suspensions were taken for analyses of wet and dry weights, protein, and radioactivity.

Collection of the Glycocalyx

Glycocalyx fractions from cell suspensions were prepared by a modification of the method of Rittenhouse et al [12]. Briefly, 10 ml cell suspension was added to 7 ml cold medium. (In the case of trypsin experiments, trypsin inhibitor (5 μg/ml) was included to prevent further proteolysis.) The suspension was then centrifuged (3,000g; 5 min) and the pellet discarded. The supernatant was then centrifuged (twice at 70,000g; 60 min) and the high-speed supernatant was analyzed for protein and radioactivity. Finally, the supernatant was dialyzed (6,000–8,000 MW cutoff) against deionized water (40–44 h; 1–4°C) and subsequently lyophilized. The glycocalyx material was assayed for protein and radioactivity.

Gel Electrophesis

Glycocalyx samples were heated in a boiling water bath for 5 min with 1% sodium dodecyl sulfate (SDS), 1% β-mercaptoethanol, and 0.5 mM EDTA before electrophoresis. SDS-polyacrylamide slab gels (8%) with stacking gel (5%) were prepared and run according

to Laemmli [16]. Gels were stained with Coomassie blue [17] and passively destained 24 h in 7.5% acetic acid.

Cell Electrophoretic Mobility

Cell suspension for each test condition was diluted 40-fold with appropriate medium for determination of surface charge density. Mobilities were measured in a microelectrophoresis apparatus using a cylindrical cell (Rank Bros., Bottisham, England) maintained at 23°C. A voltage gradient of 4.79 V/cm was applied between palladium electrodes, and by reversing the current each cell was timed to traverse 48 μ in both directions.

Surface charge density (σ) was estimated from the mobility by application of the Helmoholtz-Smoluchowski equation [18]. This estimation yields $\sigma = 12.1 \times 10^{-12}$ Eq/cm^2 for a mobility of 1 $\mu \cdot \text{sec}^{-1} \cdot \text{V}^{-1} \cdot \text{cm}$.

Analytical Procedures

Radioactivity (^{3}H) of the cell suspension and glycocalyx was assayed by liquid scintillation counting as previously described [14]. Protein analysis was carried out according to Hartree [19]. Sialic acid was determined by the method of Warren [20].

RESULTS

Exposure of Ehrlich ascites tumor cells to 25 μM $^3H_2$ DIDS for 30 min at 1–4°C results in an association of 0.062 ± 0.006 nmoles/mg dry wt (± SEM) which is not removed by the standard washing procedure. Upon resuspension in medium, cells spontaneously lose $^3H_2$ DIDS-labeled cell surface materials. Electrophoretograms of glycocalyxes collected from control (unlabeled) and $^3H_2$ DIDS-labeled cells are shown in Figure 1. There is neither a quantitative nor a qualitative difference in the protein collected in the presence or absence of $^3H_2$ DIDS labeling. This is indicative that the loss of surface material from these cells is not induced by $^3H_2$ DIDS, but rather represents a natural phenomenon.

The time course of release is shown in Figure 2. There is an initial rapid loss of radioactivity followed by a slower release up to 120 min, at which time 45.9 ± 1.2% of the cell-associated radioactivity has been released into the medium. Experiments extended to 5 h show a total loss of 72.5% of the initial bound $^3H_2$ DIDS.

Addition of trypsin (1 μg/ml) to cell suspension 120 min after resuspension leads to accelerated appearance of additional labeled material in the medium (Fig. 2). Thirty minutes after trypsin addition 71.0 ± 2.1% of total bound $^3H_2$ DIDS has been released from the cells.

Freshly $^3H_2$ DIDS-labeled cells resuspended directly into medium containing trypsin (1 μg/ml) rapidly shed cell surface material (Fig. 3), but not 100% of that bound to the cells. If it is assumed that the release of labeled cell surface material is a first-order process from a single trypsin-sensitive compartment, then the appearance of radioactivity in the medium can be described by:

$$\ln [x(t) - x(\infty)] / [x(0) - x(\infty)] = -kt \quad (1)$$

where x(0), x(t), and x(∞) are the percentages of total $^3H_2$ DIDS-labeled material released to the medium at times zero, t, and infinity, respectively, and k is the rate constant (min^{-1}) describing the release. Analysis of the data (Fig. 3) provides a best fit (r = 0.99) with k =

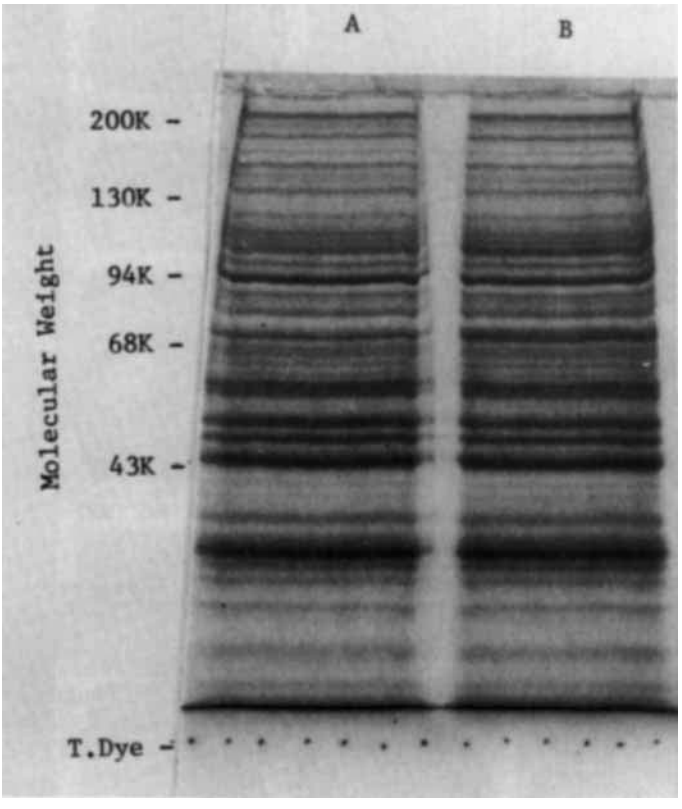

Fig. 1. Electrophoretograms of glycocalyx isolated from unlabeled control (A) and $^3H_2$DIDS-labeled (B) tumor cells. Control and $^3H_2$DIDS-labeled cells were washed and incubated (60 min at 23°C) in fresh medium to permit release of glycocalyx protein. The glycocalyx was collected and analyzed for protein distribution by SDS-polyacrylamide slab gel (8%) electrophoresis. Proteins were stained with Coomassie blue. Control cells released 0.014 mg protein per milligram cell protein in 60 min while $^3H_2$ DIDS labeled cells released 0.013 mg protein per milligram cell protein.

0.125 ± 0.012 min^{-1} (± SE) and $x(\infty) = 74.3 \pm 1.2\%$ (± SE). This is indicative that trypsin will release 74.3 ± 1.2% of the total $^3H_2$ DIDS-labeled cell surface material.

Under all of the conditions studied the maximum release of labeled material approximates 71–74% total bound $^3H_2$DIDS. Consequently, to better define the kinetics of release of the labeled surface components we have analyzed the data according to Equation 1 with $x(\infty) = 0.73 \times$ bound $^3H_2$DIDS. The spontaneous release (Fig. 2) is consistent with loss from at least two surface compartments. Extrapolation of the final four points defines a slow compartment with a half-time ($\tau_{1/2}$) of 360 min, which represents 45% of the labile material (33% of total bound $^3H_2$DIDS). Subtraction of this compartment from the total loss reveals a second compartment ($\tau_{1/2}$ = 20 min) which represents 36% of the labile pool (26% of total bound $^3H_2$DIDS). The remaining (14% total bound) labile material may represent a very rapid initial loss of labeled material which occurs upon resuspension of the cells.

Trypsin, added after 120 min of spontaneous release, converts the kinetics to that of a single labile pool with $\tau_{1/2}$ = 7.2 min. A similar result is obtained for loss from cells resus-

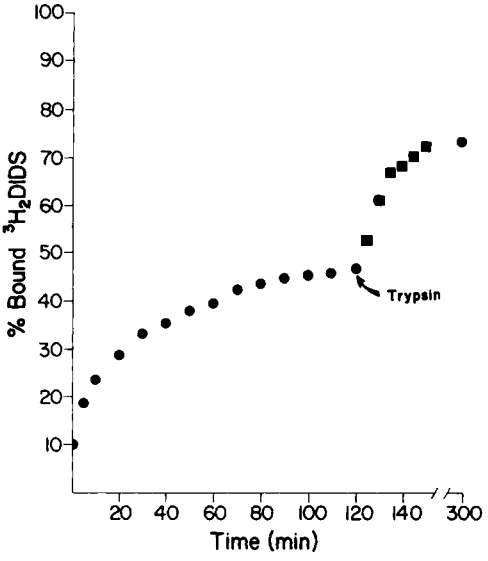

Fig. 2. Release of $^3H_2$DIDS-labeled cell surface components. Cells were exposed to $^3H_2$DIDS (25 μm) for 30 min at 1–4°C, then washed free of $^3H_2$DIDS and resuspended in fresh medium at 23°C. Aliquots of the cell suspension were removed at specified times (1–120 min and 5 h) and assayed for radioactivity in the medium. Release to the medium is reported as percentage of the initial total binding. Values shown (●) are the averages for nine experiments. In four of these experiments, trypsin (1 μg/ml) was added to cell suspension after 2 h of spontaneous release and the release of bound $^3H_2$DIDS was followed for 30 min (■). Standard errors of the mean were smaller than the symbols and are not shown.

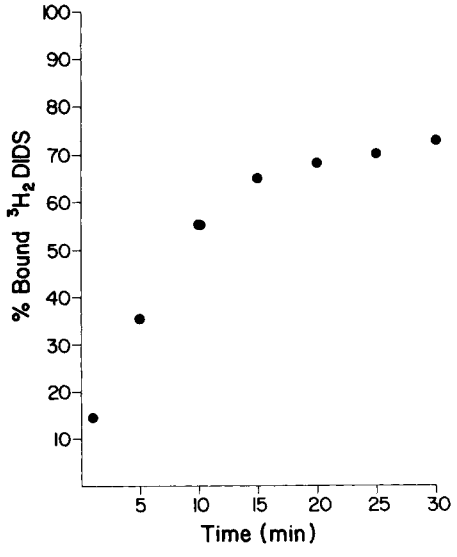

Fig. 3. Effect of trypsin on release of $^3H_2$ DIDS-labeled cell surface components. Cells were exposed to $^3H_2$ DIDS as before (Fig. 1) and resuspended in fresh medium containing trypsin (1 μg/ml) at 23°C. Aliquots of the cell suspension were removed at specified times (1–30 min) and assayed for radioactivity in the medium. Release to the medium is reported as percentage of the total initial $^3H_2$ DIDS bound. Values are averages for four experiments. Standard errors of the mean were smaller than the symbols and are not shown.

pended initially into trypsin. In this case the trypsinization results in a slightly accelerated release of surface label ($\tau_{1/2}$ = 5.5 min).

Glycocalyx Fraction

The glycocalyx fractions collected from cell suspensions used for the kinetic studies were assayed for protein and radioactivity. The results are given in Table I.

Glycocalyx collected from cell suspension (10 ml) incubated for 2 h in medium contains 2.18 ± 0.15 mg protein (1.88 ± 0.23% total cell protein) with a specific activity of 3.85 ± 0.53 × 10^6 cpm/mg protein. This represents a 19.7-fold enrichment of $^3H_2$DIDS label compared to total cell protein. Resuspension of these cells in trypsin-containing medium (1 μg/ml; 30 min) results in an additional release of protein of 2.47 ± 0.23 mg (2.32 ± 0.29% total cell protein) having specific activity (1.50 ± 0.16 × 10^6 cpm/mg protein).

Glycocalyx derived from cells exposed to trypsin for 1 h immediately after $^3H_2$DIDS labeling contains 250% more protein than comparable control cells. While the specific activity of glycocalyx from trypsin-treated cells in 83% of control, the difference is not significant for three experiments. However, in each of the experiments the specific activity of control glycocalyx exceeded that after trypsinization.

In a single experiment we compared the sialic acid contents of glycocalyx collected from spontaneous and trypsin-induced release to that released by neuraminidase (100 units/ml). Glycocalyx collected from control cells contained no detectable sialic acid, while trypsinization resulted in glycocalyx containing 12.5% of the sialic acid available to neuraminidase treatment (0.20 nmoles/mg dry wt versus 1.60 nmoles/mg dry wt).

Cell Electrophoretic Mobility and Surface Charge Density

The electrophoretic mobilities of Ehrlich ascites tumor cells in these studies are shown in Figure 4. The mobiliy of cells washed free of ascitic fluid with medium is 0.86 ± 0.01 μ·sec^{-1}·V^{-1}·cm (±SE) (σ = 1.06 × 10^{-11} Eq/cm^2). An additional wash with medium contain-

TABLE I. Collection of Glycocalyx

Experimental procedure	Protein recovered in supernatant, mg (% cell protein)	SA: Protein recovered in supernatant (cpm/mg protein × 10^{-6})	SA: Total cell protein (cpm/mg protein × 10^{-6})
A. Consecutive Collection (2)[a]			
Control	2.18 ± 0.15 (1.88 ± 0.23)	3.85 ± 0.53	0.20 ± 0.02
Experimental	2.47 ± 0.23 (2.32 ± 0.29)	1.50 ± 0.16	0.09 ± 0.01
B. Simultaneous Collection (3)[a]			
Control	1.08 ± 0.22 (0.88 ± 0.01)	4.21 ± 0.39	0.15 ± 0.03
Experimental	2.65 ± 0.60 (2.16 ± 0.23)	3.50 ± 0.46	0.15 ± 0.03

Collection of glycocalyx. Cells were exposed to $^3H_2$DIDS (25 μM) for 30 min at 1–4°C, then washed with 0.5% albumin-containing medium. A: Consecutive collection (10 ml cell suspension): Cells were resuspended in fresh medium for 2 h and the supernatant collected (control). The cells were then resuspended in fresh medium plus trypsin (1 μg/ml) for 30 min and the resulting supernatant was again collected (experimental). B: Simultaneous collection: Cell suspension was divided and the cells (10 ml cell suspension) incubated in the absence (control) or presence (experimental) of trypsin (1 μg/ml) for 1 h prior to glycocalyx collection. The supernatants were analyzed for total protein and radioactivity.

[a]Number of experiments. Standard errors of the mean are given.

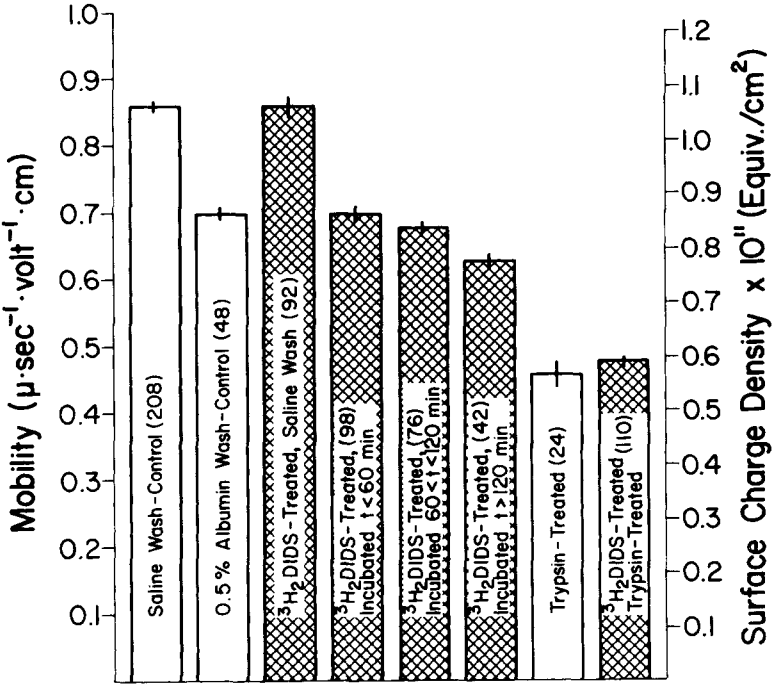

Fig. 4. Effect of $^3H_2$DIDS binding and release on cell electrophoretic mobility and surface charge density. Conditions describing the cell treatment are given in each bar. Cell suspensions were diluted 40-fold with the appropriate media and the electrophoretic mobility was determined at 23°C. Number of determinations for each condition is given in parentheses. Standard errors of the mean are shown.

ing 0.5% albumin reduces the mobility to 0.70 ± 0.01 $\mu \cdot sec^{-1} \cdot V^{-1} \cdot cm$. To determine whether the reduction in mobility after albumin wash reflected an actual loss of surface charge or simply "masking" by absorbed albumin, we determined the mobility of cells washed with medium and resuspended in medium containing 0.5% albumin. The mobility (not shown) was not different from saline-washed cells.

Labeling of cells with $^3H_2$DIDS followed by either medium or albumin-containing wash does not alter the cell mobility compared to the appropriate control. During the first or second hour of spontaneous glycocalyx loss there is no significant change in mobility. However, cells which have been incubated for 2 h (plus 30 min for measurements) have a significantly ($P < 0.05$) reduced mobility (0.63 ± 0.02 $\mu \cdot sec^{-1} \cdot V^{-1} \cdot cm$).

Trypsin treatment of unlabeled cells washed with either medium or 0.5% albumin-containing medium reduces the mobility by 35%. The presence of bound $^3H_2$DIDS does not alter the effect of trypsin on cell mobility.

DISCUSSION

These studies demonstrate that incubation of Ehrlich ascites tumor cells with $^3H_2$DIDS (25 μM; 30 min) at 1–4°C results in an irreversible binding of this agent (0.062 ± 0.006 nmoles/mg dry wt) to the cell surface. Previous studies from our laboratory established that the $^3H_2$DIDS associated with the cell using identical conditions was confined to the surface

and did not enter the cells. Furthermore, the cells maintain viability as judged by Trypan blue exclusion (> 96%) and capacity to accumulate amino acid [14].

Resuspension of labeled cells in fresh medium at 23°C resulted in the spontaneous release of surface proteins into the medium (Fig. 2). Kinetic analysis of this process indicates the existence of at least three major binding populations. Approximately 27% of the bound $^3H_2$DIDS is associated with components of the cell which are not released spontaneously or by mild trypsination. The remaining 73% of bound $^3H_2$DIDS is spontaneously released from two major labeled compartments representing 33% ($\tau_{1/2}$ = 360 min) and 26% ($\tau_{1/2}$ = 20 min) of the total binding, respectively.

The existence of two kinetically dissimilar surface compartments does not imply that only two chemical components are spontaneously lost by the cell. The sensitivity of the kinetic analysis does not permit the separation of components which have similar rates of loss. Chemical analysis [12] and polyacrylamide gel electrophoresis [Fig. 1] of glycocalyx derived from Ehrlich ascites tumor cells have shown the presence of a complex mixture of proteins and glycosaminoglycans. Thus it is likely that each of the kinetically identifiable compartments represents a mixture of surface macromolecules which are shed with similar rates.

One possible explanation for the different rates of release is a combination of surface shedding and internalization of cell membrane with subsequent secretion [21]. However, the effect of mild trypsination on the release of labeled material argues against this mechanism. All of the labile $^3H_2$DIDS-labeled components are readily accessible to trypsin. Since trypsin at the concentration used does not alter cell viability, it is unlikely that it could accelerate the release of internalized membrane components destined for secretion.

The most likely explanation for these findings is that the two labile compartments are spontaneously shed into the environment at different rates. Trypsin has access to both and hydrolyzes them at similar rates. This is reflected by conversion of the kinetics of release to that of a single population.

It is not possible in the present study to identify the source of the shed material. However, Rittenhouse and co-workers [22] have recently shown that approximately 30% of the total protein which is spontaneously shed by these cells is in the form of high-molecular weight aggregates which contain host-derived, tumor-associated immunoglobulin G (TAIg) and a complement component (C3). Numerous tumor-derived glycoproteins are present in shed material, but only one of these (45K) is associated with the host-derived components [23]. Thus, our finding of two kinetic compartments would be consistent with a combined process of release of immune complexes and natural cell surface turnover.

The bound $^3H_2$DIDS (27%) which is not subject to either spontaneous release or trypsin hydrolysis may represent an association with surface components inaccessible to trypsin or binding interaction at a site distal to trypsin hydrolysis. In the erythrocyte, H_2DIDS interaction with the anion transport protein occurs at a site interior to cleavage by trypsin [24]. Similarly, anion transport inhibition by H_2DIDS in Ehrlich ascites cells is not relieved by spontaneous loss of the glycocalyx [25], suggesting that H_2DIDS binds to protein components of the cell membrane which are not labile.

Comparison of the glycocalyxes prepared by trypsinization of freshly labeled cells and cells which have been incubated for 2 h prior to trypsin treatment is revealing. Both treatments release equivalent amounts of protein (Table I) and result in the same total loss of bound $^3H_2$DIDS (Figs. 2 and 3). However, the specific activity of glycocalyx from cells which had spontaneously shed material prior to trypsin treatment is only 43% of that from freshly labeled cells. This reduction in specific activity might result from an exposure

of trypsin-sensitive protein previously masked by the glycocalyx. Since trypsin itself effectively removes the $^3H_2$DIDS-labeled labile components of the glycocalyx within 30 min, the same exposure should occur. If spontaneous release unmasks cryptic protein, we would expect an extended exposure to trypsin to hydrolyze at least an equivalent amount of protein. However, the total protein collected after 2 h of spontaneous release plus 30 min in trypsin exceeds that removed by 1 h in trypsin. This makes an unmasking effect unlikely, in agreement with previous conclusions based on access of the cell surface to iodination [12].

Consequently, the reduction in specific activity after spontaneous release and the increase in total protein subject to spontaneous release plus trypsin suggest that new cell surface material has been incorporated into the glycocalyx during the incubation period (2 h). This suggestion is in concert with the view that surface membrane components are constantly renewed by insertion of newly synthesized molecules to replace those shed into the environment [26]. If this is the case, the kinetic experiments reported here provide an estimate of the rates of turnover of the glycocalyx components.

In an effort to further characterize the interaction of $^3H_2$DIDS with the cell surface, we have examined the effects of labeling and subsequent removal on the cell electrophoretic mobility. Ehrlich ascites tumor cells carry a net negative surface charge. Much of the surface negativity can be abolished by neuraminidase treatment [27], indicating an important contribution from bound sialic acid. In addition, it has been suggested that a large portion of the cellular, negatively charged glycosaminoglycans are located at the cell periphery [12].

In the present studies, the binding of $^3H_2$DIDS to the cell surface is without effect on surface charge density. Subsequent spontaneous loss of the glycocalyx does not alter mobility during the initial phase (Fig. 4). However, after 2 h of incubation there is a significant decline in surface charge density. This temporal pattern of surface charge change can be compared to the kinetics of spontaneous $^3H_2$DIDS label release, suggesting that only the more slowly released population ($\tau_{1/2}$ = 360 min) contributes to the alteration in cell surface charge.

The glycocalyx collected by spontaneous release from these cells contains no sialic acid [12]. Others have demonstrated the presence of glycosaminoglycans in glycocalyx collected by gentle elution [12]. It seems likely, then, that the charge-bearing, slowly released surface material contains significant amounts of glycosaminoglycans.

Trypsin treatment of the cells further reduces the surface charge density and hydrolyzes the labile compartments of the cell surface (Figs. 2 and 3). Glycocalyx collected from these cells contains 12.5% of the sialic acid which can be released by neuraminidase. Whether the sialic acid-containing glycoproteins which are susceptible to trypsin (1 μg/ml) contributed the cell mobility is not clear. Trypsin, at the concentration used, readily removes glycosaminoglycans from the cell surface [28]. This is consistent with both glycoproteins and glycosaminoglycans contributing to the altered mobility, but does not necessarily indicate that sialic acid-associated glycoproteins constitute part of the slowly desorbed surface components.

Taken together, the results of this investigation demonstrate the applicability of $^3H_2$DIDS as a label for cell surface constituents. $^3H_2$DIDS irreversibly interacts with three major populations of cell surface components. One of these does not dissociate from the surface during the time course of the experiments. The other two are spontaneously released by the cell with measurably different rates. It is likely that this represents different rates of surface protein turnover. Alterations in the cell surface charge density suggest that the more slowly desorbing of these labile compartments is rich in the glycosaminoglycans of the cell surface.

ACKNOWLEDGMENTS

This study was supported by grant CA 10917, US Public Health Service, National Cancer Institute.

We gratefully acknowledge the excellent technical assistance of Mrs. Rebecca Corcoran, Ms. Ellen Edwards, and Mrs. Carmen DuBose.

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