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NADH oxidase activity of HeLa plasma membranes inhibited by the antitumor sulfonylurea *N*-(4-methylphenylsulfonyl)-*N'*-(4-chlorophenyl)urea (LY181984) at an external site

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Abstract

NADH oxidase activity from HeLa plasma membranes was inhibited by the antitumor sulfonylurea *N*-(4-methylphenylsulfonyl)-*N'*-(4-chlorophenyl)urea (LY181984). With sealed right side-out vesicles, the drug inhibited half maximally at about 30 nM and the inhibition was nearly complete. A closely related but growth-inactive sulfonylurea, *N*-(4-methylphenylsulfonyl)-*N'*-(phenyl)urea (LY181985), did not inhibit the activity. With plasma membranes first solubilized with 2% Triton X-100, activity also was inhibited by LY181984 and not by LY181985 but the maximum inhibition at 10 μM LY181984 was only 50%. When sealed right side-out plasma membrane vesicles were frozen and thawed repeatedly to evert some of the vesicles into an inside-out configuration, the NADH oxidase activity again was only about 50% inhibited by 1 μM LY181984. In such preparations, the right side-out vesicles exhibited an electrophoretic mobility greater than that of the inside-out vesicles. Sidedness was confirmed by measurements of ATPase latency and binding of immunogold-labeled concanavalin A. When the two vesicle populations were resolved by preparative free-flow electrophoresis, the active antitumor sulfonylurea LY181984 inhibited only the NADH oxidase activity of the right side-out vesicles. These findings suggested two NADH sites or activity isoforms for the plasma membrane NADH oxidase. One activity, inhibited by LY181984, appeared to be accessible to external NADH only with sealed right side-out vesicles. The other, not inhibited by LY181984, was accessible to NADH only with inside-out vesicles or after membrane disruption by Triton X-100. The findings demonstrate that the NADH oxidation site inhibited as a result of binding the active antitumor sulfonylurea LY181984 is at the external cell surface. Plasma membrane vesicles from HeLa cells are able to oxidize NADH supplied to either membrane surface but only with inside-out vesicles is NADH oxidation sensitive to inhibition by the antitumor sulfonylurea.

Keywords: NADH oxidase activity; Inhibition; Sulfonylurea; Antitumor drug; Plasma membrane; HeLa cell

1. Introduction

We previously reported that HeLa plasma membranes bind the active antitumor sulfonylurea LY181984 with high affinity (K_d of ca. 30 nM) [1]. Upon sulfonylurea binding, the plasma membrane vesicles of HeLa cells exhibited a sulfonylurea-inhibited oxidation of NADH [2]. The latter was inhibited with an ED_{50} of about 30 nM. The inhibition was analyzed kinetically and found to be uncompetitive [2].

In this report, we demonstrate that plasma membranes of HeLa cells contain at least two NADH oxidase activities. One is inhibited by the antitumor sulfonylurea, the other is not. For these studies, sulfonylureas active and inactive in the inhibition of growth of in vivo murine solid tumors [3,4] were provided by Lilly Research Laboratories (Indianapolis). The compounds have a high degree of efficacy and a relatively low toxicity [4,6]. Of these, one active and one inactive in inhibiting growth of HeLa cells in parallel to that of the murine tumors [5], were selected for detailed study.

To explore the orientation within the plasma membrane

Abbreviations: Con A, concanavalin A; LY181984, *N*-(4-methylphenylsulfonyl)-*N'*-(4-chlorophenyl)urea; LY181985, *N*-(4-methylphenylsulfonyl)-*N'*-(phenyl)urea.

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of the two forms of NADH oxidase activity, sealed right side-out HeLa plasma membrane vesicles were treated to yield a mixture of right side-out and inside-out vesicles [7]. The two populations were separated by preparative free-flow electrophoresis and sidedness was verified using well-established marker criteria. Only when NADH, an impermeant substrate, was supplied to right side-out vesicles was NADH oxidation inhibited by the sulfonylurea. Taken together with detergent solubilization experiments, the results provide evidence for two forms of NADH oxidase associated with the plasma membranes of HeLa cells, one external and inhibited by the active antitumor sulfonylurea and one internal and resistant to inhibition by the active antitumor sulfonylurea.

2. Materials and methods

2.1. Growth of cells

HeLa S cells were grown on minimal essential medium (S-MEM) (Jolik modified) with glutamine (244 mg/l) and phosphate (1.3 g/l Na_2HPO_4) and without $CaCl_2$ plus 5% donor horse serum. Gentamycin sulfate (50 mg/l) and sodium bicarbonate (2 g/l) were added. Cells were collected by centrifugation for 6 to 15 min at 1000 to 3000 rpm (e.g., 6 min at 3000 rpm or 15 min at 1000 rpm).

2.2. Purification of plasma membranes from HeLa cells

HeLa cells grown as suspension cultures were collected by centrifugation for 6 min at 3000 rpm. The cell pellets were resuspended in 0.2 mM EDTA in 1 mM $NaHCO_3$ in an approximate ratio of 1 ml per 10^8 cells and incubated on ice for 30 min to swell the cells. Homogenization was with a Polytron homogenizer for 40 s at 10 500 rpm using an ST-probe and 8 ml aliquots. Cell breakage was at least 90% without breakage of nuclei as determined by light microscopy.

The homogenates were centrifuged for 10 min at $175 \times g$ to remove unbroken cells and nuclei. The supernatant was centrifuged a second time at $1.4 \cdot 10^6 \times g$ min to prepare a plasma membrane-enriched fraction. Plasma membrane-enriched pellets were resuspended in 0.2 M potassium phosphate buffer in a ratio of 1 ml per pellet from approx. $5 \cdot 10^8$ cells. The resuspended membranes were then loaded onto the two-phase system constituted on a weight basis and consisting of 6.5% (w/w) Dextran T-500 (Pharmacia), 6.4% (w/w) poly(ethylene glycol) 3350 (Fisher) in 5 mM potassium phosphate buffer, pH 7.2 [8]. The resuspended microsomal pellet (1 g) was added to the two-phase system and the weight of the system was brought to 8 g with distilled water. The tubes were inverted vigorously for 40 times in the cold (4°C) and the phases separated by centrifugation at $150 \times g$ for 5 min. The upper phases were withdrawn with a pasteur pipette, diluted with 5 volumes of cold 1 mM $NaHCO_3$ and the membranes were collected by centrifugation. The purity of the plasma membrane was determined to be > 90% by electron microscope morphometry. The yield was 2 mg plasma membrane protein from 10^9 cells.

2.3. Spectrophotometric assay

NADH oxidase activity was determined as the disappearance of NADH measured at 340 nm in a reaction mixture containing 25 mM Tris-Mes buffer, pH 7.2, 1 mM KCN to inhibit any potential mitochondrial oxidase activity, and 150 μM NADH at 37°C with constant stirring. Activity was measured using a Hitachi U3210 with continuous recording over 5 or 10 min intervals. A millimolar extinction coefficient of 6.22 was used to determine NADH disappearance.

2.4. Right side-out vs. inside-out membrane vesicles

For preparation of right side-out and inside-out membrane vesicles, sealed right side-out HeLa plasma membrane vesicles were subjected to a series of freeze-thaw treatments followed by hypotonic lysis facilitated by drawing the suspended vesicles through a fine hypodermic needle. This procedure everted some of the vesicles into a cytoplasmic side-out configuration [7]. The cytoplasmic side-out vesicles lacked the strongly negatively charged surface molecules that characterize right side-out vesicles and the two populations were readily resolved by preparative free-flow electrophoresis [7].

Free-flow electrophoresis separations were as described [9]. The mixture of right side-out and inside-out vesicles was suspended in chamber buffer (see below). The equipment was a VAP-22 continuous free-flow electrophoresis unit (Bender and Hobein, Munich, Germany). The electrophoresis medium (chamber buffer) consisted of 0.25 M sucrose, 2.0 mM KCl, 10 mM triethanolamine, and 10 mM acetic acid, pH 6.5 (NaOH). The electrode buffer contained 100 mM triethanolamine and 100 mM acetic acid, pH 6.5 (NaOH). The separations were carried out at 4°C under a constant voltage of 1150 V over the 9.2 cm field with a resulting current of 180 ± 5 mA. The buffer flow was 1.7 ml/fraction/h and the sample injection was 2.7 ml/h. The separation profile was monitored by collecting individual fractions and monitoring absorbance at 280 nm. The membranes were collected from pooled fractions by centrifugation for 30 min at $95\,000 \times g$ (Beckman SW-28).

2.5. ATPase latency assays to verify vesicle orientation

An assay of total ATPase activity was conducted in a 500 μl reaction volume containing 25 mM Tris-Mes, pH 6.5, 1 mM NaN_3, 1 mM sodium molybdate, 0.1 mM EDTA, 3.0 mM $MgSO_4$, 3.0 mM disodium ATP, 50 mM KCl, and 5 to 10 μg plasma membrane protein. Reactions

were incubated at room temperature for 30 min and stopped by addition of 50 μl 25% TCA and 300 μl 1% SDS. The released phosphate was determined by addition of 2.5 ml freshly prepared reagent containing 0.8% ammonium molybdate, 0.27% ascorbic acid, and 0.045% $SnCl_2$ in 1 N H_2SO_4. The absorbance at 723 nm was measured after 30 min.

Protein was measured by the bicinchoninic acid procedure [10] with bovine serum albumin as the standard.

2.6. Concanavalin A (Con A) binding

The membrane pellets were washed twice with 1.5 ml of 5 mM Hepes-KOH, pH 7.2, containing 0.25 M sorbitol (to approximate the concentration of sucrose in the free-flow electrophoresis buffer) by centrifugation in a microfuge (Eppendorf) for 5 min. The pellets were resuspended in the same buffer to give a 100 μg protein/100 μl solution. An equal volume of a solution of 10 μg Con A conjugated with 20 nm immunogold (Sigma)/100 μl (10% of total protein) was added to the membrane suspensions. After incubation for 40 min at room temperature, 1.5 ml of 2.5% glutaraldehyde in 0.1 M sodium phosphate, pH 7.2, followed by centrifugation for 5 min in the microfuge. The supernatant was discarded, fresh 2.5% glutaraldehyde in phosphate buffer, pH 7.2, was added, and the material was processed for electron microscopy.

2.7. Electron microscopy

Small wedges cut from membrane pellets fixed with 2.5% glutaraldehyde in 0.05 M sodium phosphate, pH 7.2, were rinsed three times with 0.05 M sodium phosphate, pH 7.2, and post-fixed with 1% osmium tetroxide in 0.05 M sodium phosphate, pH 7.2, overnight at 4°C. Samples were

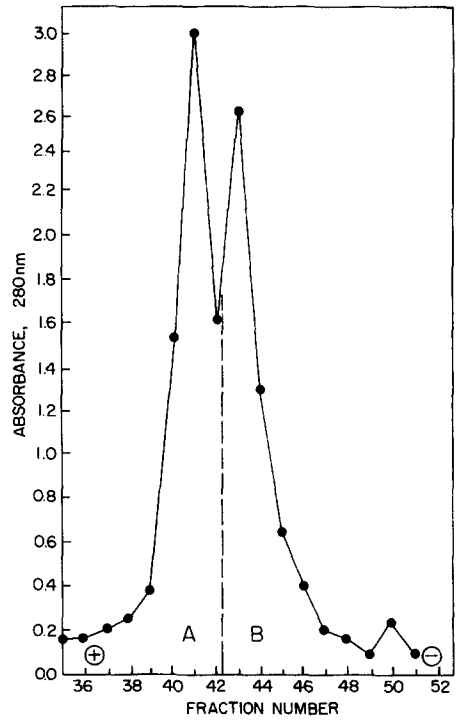

Fig. 2. Absorbance profiles at 280 nm of free-flow electrophoretic separations of HeLa cell plasma membranes. Membranes prepared by aqueous two-phase partition were frozen and thawed and passed through a narrow gauge needle to invert some of the vesicles. The right side-out vesicles (A) were then separated from the cytoplasmic side-out vesicles (B) by preparative free-flow electrophoresis.

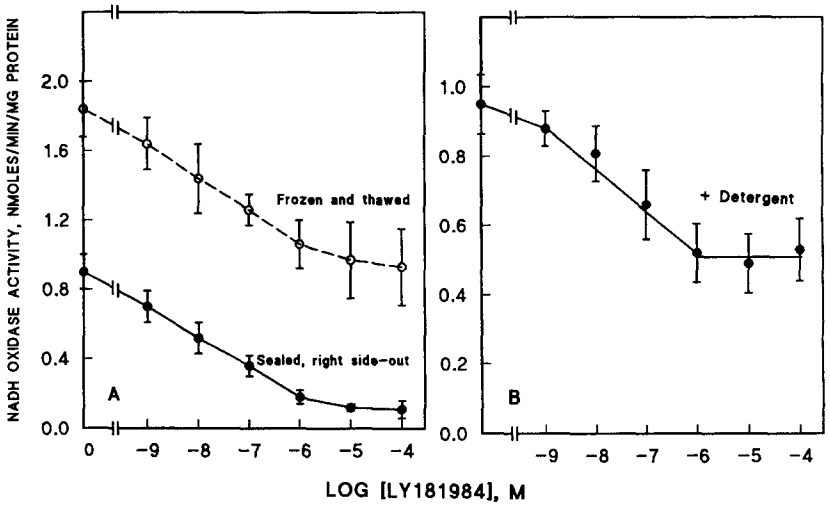

Fig. 1. NADH oxidase activity of HeLa plasma membranes with increasing concentrations of LY181984. (A) Solid symbols and lines (lower curve). Sealed right side-out vesicles of HeLa plasma membranes. The antitumor sulfonylurea LY181984 active in inhibition of tumor growth resulted in nearly complete inhibition of NADH oxidase activity. Open symbols, dashed lines (upper curve). With vesicles of plasma membranes that were repeatedly frozen and then thawed, the NADH oxidase was only about 50% inhibited by LY181984. (B) HeLa plasma membranes solubilized with 0.1% Triton X-100. Inhibition of NADH oxidase by the antitumor active LY181984 (solid symbols) was about 50%. For each experiment duplicate determinations were averaged. Results are averages from three experiments ± S.D. among experiments.

dehydrated in an acetone series, and embedded in Epon [11]. Lead-stained thin sections were examined and photographed with a Philips EM/200.

3. Results

The NADH oxidase activity of vesicles of plasma membranes of HeLa cells is inhibited by the antitumor sulfonylurea active in growth inhibition, *N*-(4-methylphenylsulfonyl)-*N'*-(4-chlorophenyl)urea (LY181984) (Fig. 1). The degree of maximum inhibition, however, varied from nearly complete (Fig. 1A) to about 50% (Fig. 1A,B) depending on whether or not vesicles were pretreated to expose both external and internal membrane surfaces.

With sealed right side-out vesicles, the NADH oxidase was nearly completely inhibited by LY181984 (Fig. 1A, lower curve). Half maximal inhibition was at about 30 nM LY181984 and maximum inhibition was achieved at about 1 μM.

When plasma membrane preparations that had been repeatedly frozen and thawed were analyzed, some of the vesicles became everted with the result being a mixture of vesicles of smaller diameter some of which were right side-out and some of which were inside-out. When such preparations were assayed for NADH oxidase activity, the activity was still inhibited by LY181984 with near maximum inhibition at about 1 μM. However, on average, the maximum inhibition was only about 50% (Fig. 1A, upper curve) as compared to > 90% for the sealed, right side-out vesicles of Fig. 1A (lower curve).

LY181984 inhibited as well in detergent-solubilized preparations of plasma membranes from HeLa cells (Fig. 1B). As with frozen and thawed vesicles, the inhibition by LY181984 was only about 50%. That the inhibition of the activity by LY181984 in detergent-solubilized preparations was only 50% compared to intact membranes suggested that there might be at least two NADH sites or activity isoforms associated with the plasma membrane, one inhibited by sulfonylurea and the other not inhibited.

With each of the different plasma membrane preparations of Fig. 1A and Fig. 1B, a chemically related but growth inactive sulfonylurea, *N*-(4-methylphenylsulfonyl)-*N'*-(phenyl)urea (LY181985), was tested at the same concentrations as for LY181984. Within the standard deviations among the two preparations analyzed (± 10%), the LY181985 was without effect (data not shown).

NADH, being impermeant, can reach an outside site

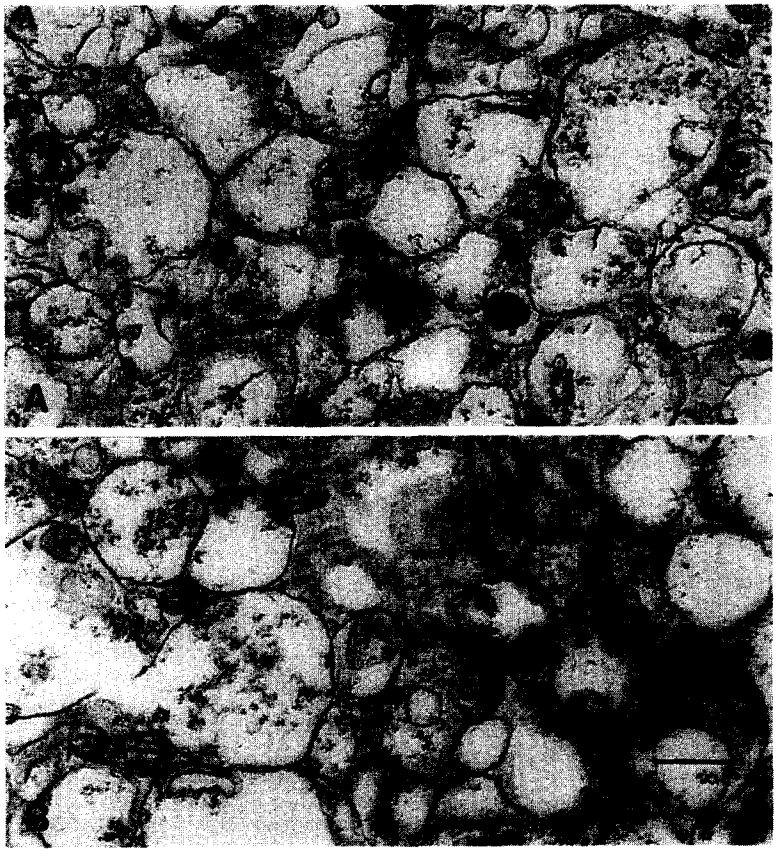

Fig. 3. Electron micrographs of HeLa plasma membrane fractions purified by aqueous two-phase partition and separated by preparative free-flow electrophoresis into two fractions of differing electrophoretic mobility. (A) Electrophoretic fraction of greatest mobility and high ATPase latency consisting of right side-out vesicles. (B) Electrophoretic fraction of lesser mobility and low ATPase latency consisting of inside-out vesicles. Both classes of vesicles appeared to be sealed. Scale bar = 0.5 μm.

only with right side-out vesicles and would be accessible to an inside site only with inside-out vesicles. To test the suggestion that the sulfonylureas inhibit only the oxidase activity exposed to NADH with right side-out vesicles, i.e., an external NADH site, HeLa cell plasma membranes were subfractionated into separate populations of inside-out and right side-out vesicles. To effect this separation, a HeLa cell plasma membrane preparation obtained by aqueous two-phase partition was frozen and thawed several times and a portion of the vesicles were everted by passing them through a narrow gauge needle. The right side-out vesicles carried a net negative surface charge greater than that of the inside-out vesicles and thus were resolved by preparative free-flow electrophoresis as illustrated in Fig. 2. The plasma membrane fractions of greatest electrophoretic mobility, fractions 38–42 (A), were combined as representing right side-out vesicles and fractions 43 to 47 (B) of lesser electrophoretic mobility were combined as representing inside-out vesicles.

Electron microscopy of the isolated vesicles revealed relatively uniform populations of closed vesicles following free-flow electrophoretic separations (Fig. 3). More than 90% of the vesicles had morphological characteristics of plasma membranes. The membrane vesicles were large, smooth (lacking ribosomes or electron transport particles) and were 9 to 10 nm in thickness. Additionally, the plasma membranes when contrasted with lead showed clearly the dark-light-dark pattern characteristic of the plasma membrane. There was little or no perceptible contamination by endoplasmic reticulum (6 nm thick membranes, attached

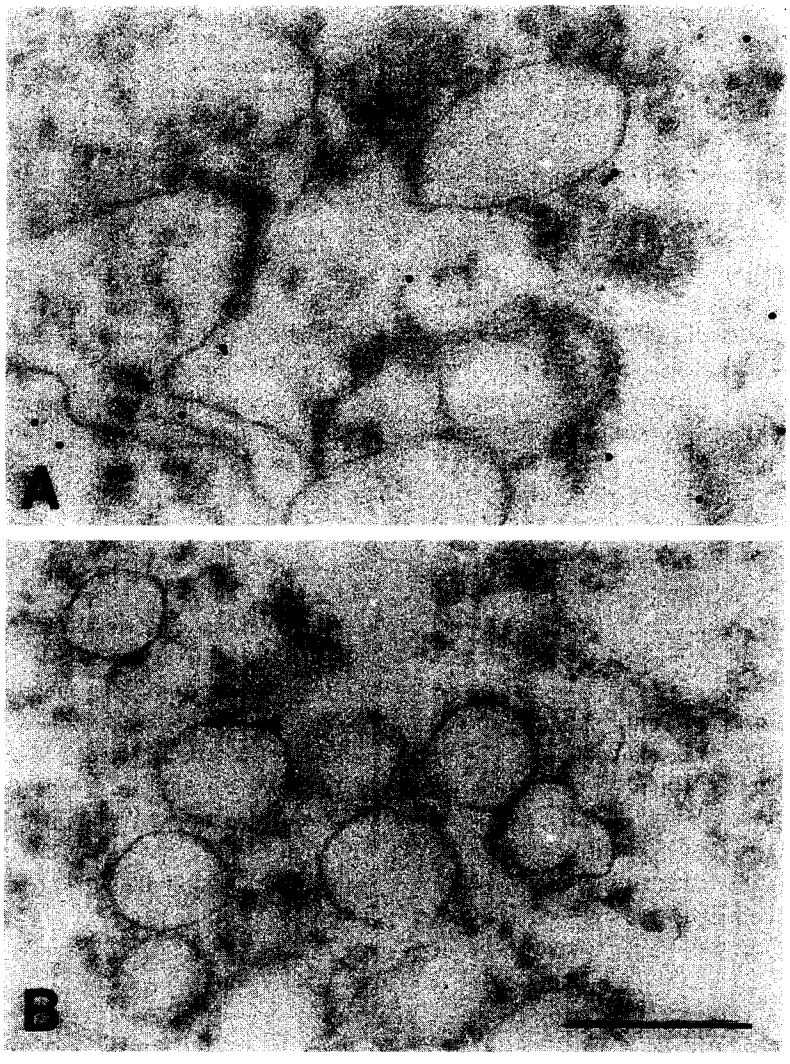

Fig. 4. Binding of 20 nm immunogold-labeled concanavalin A to purified plasma membranes of HeLa cells. Membrane vesicles were isolated by aqueous two-phase partition and freeze-thaw disruption was used to evert some of the vesicles from a right side-out to an inside-out configuration. Subsequent separation was by preparative free-flow electrophoresis fractions enriched in right side-out and inside-out vesicles. By reacting with surface glycoproteins, the external plasma membrane surface was marked. (A) Vesicles of electrophoresis fractions 38–42 bound concanavalin A and were right side-out (cytoplasmic side-in). (B) Vesicles of electrophoresis fractions 43–47 bound concanavalin A poorly and were inside-out (cytoplasmic side-out). Of the vesicles in the right side-out fraction > 90% were marked by concanavalin A. Of the vesicles in the inside-out fraction < 10% were marked by concanavalin A. Scale bar = 0.5 μm.

ribosomes), mitochondria (cristae and f_1/f_o-ATPase), Golgi apparatus (stacked cisternae) or nuclear envelope (presence of chromatin and 6 nm membranes with pores). The appearance of the right side-out membranes (Fig. 3A) and of the inside-out membranes (Fig. 3B) was similar except that cytoplasmic debris (e.g., free ribosomes) was located dominantly on the outside of the right side-out vesicles (Fig. 3A) but frequently appeared to be contained within the inside-out vesicles (Fig. 3B) as might be expected from accidental entrapment during the eversion process.

To determine sidedness, the latency of ATP activity was determined. Both the starting vesicles and the more electrophoretically mobile right side-out vesicles exhibited only low ATPase activity unless detergent was added to disrupt the vesicles (Table 1). In contrast, the inside-out vesicles exhibited the same high ATPase activity both before and after detergent-disruption of the membranes.

Orientation of the vesicles in the two different electrophoretic fractions was determined from concanavalin A binding visualized by electron microscopy using concanavalin A conjugated with 20 nm immunogold (Fig. 4). The initial plasma membrane isolates from two-phase partition were mixtures of vesicles reacting strongly and of vesicles reacting weakly with immunogold-linked to concanavalin A. However, with vesicles first separated into two populations by preparative free-flow electrophoresis, the strongly-reactive vesicles were concentrated in fraction A of the more electronegative part of the separation (Fig. 4A) whereas the weakly reactive vesicles were concentrated in fraction B in the least electronegative part of the separation (Fig. 4B). Latency of concanavalin A inhibition of 5′-nucleotidase was exhibited by vesicles of fraction B but not by vesicles of fraction A. These findings suggest that the two populations of plasma membrane vesicles obtained by preparative free-flow electrophoresis (Fig. 4) resulted from the presence of both inside-out and right side-out plasma membrane vesicles and their subsequent resolution by free-flow electrophoresis.

When right side-out and cytoplasmic side-out plasma membrane vesicles from HeLa plasma membranes were compared, the inhibition of NADH oxidase activity by the active antitumor sulfonylurea LY181984 was seen only with the right side-out vesicles (Fig. 5, solid symbols). The dose response was equivalent to that observed with the starting preparations of sealed right side-out vesicles (Fig. 1A, lower curve). Inhibition was > 90% complete and

Table 1
ATPase latency of HeLa plasma membrane (μmol P_i /h/mg protein)

Fraction	0.2% Triton X-100	
	−	+
Fraction A right side-out	< 0.5	6.4
Fraction B inside-out	5.5	7.1

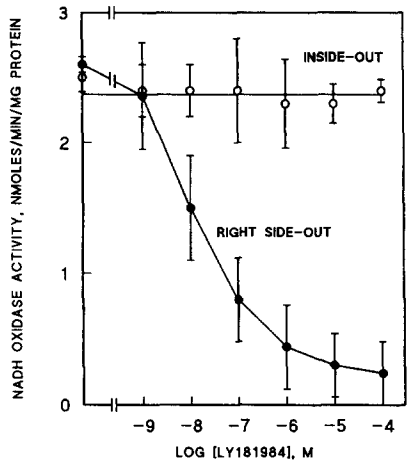

Fig. 5. NADH oxidase activity of free-flow electrophoretic separations of inside-out (solid symbols) and right side-out plasma membrane vesicles (open symbols) in response to the logarithm of the concentrations of the active antitumor sulfonylurea LY181984. The NADH oxidase activity of the more electrophoretically mobile fractions corresponding to fraction A (electrophoresis fractions 38–42) of Fig. 5 was inhibited by the sulfonylurea whereas the activity of less electrophoretically mobile fractions corresponding to fraction B (electrophoresis fractions 43 to 47) of Fig. 5 was unaffected by the antitumor sulfonylurea. Values are averages of duplicate determinations for each of three different experiments ± S.D. among experiments.

near maximal at 1 μM. The concentration for half-maximal inhibition was about 30 nM. In contrast, the fraction enriched in inside-out vesicles did not respond to LY181984 (Fig. 5, open symbols).

Comparing the same fractions as analyzed for Fig. 5, the right side-out vesicles that responded to the active antitumor sulfonylurea, LY181984, again did not respond to the growth inactive sulfonylurea analog LY181985 (data not shown). An overall inhibition of about 20% at 10^{-4} M LY181985 was observed with the inside-out vesicle fractions.

Intact HeLa cells oxidized NADH in the absence of added external oxidants at rates comparable to those exhibited by isolated plasma membranes. This oxidation of NADH via an external plasma membrane site also was inhibited by the growth-active antitumor sulfonylurea LY181984 but not by LY181985 (data not shown). These findings provide additional support for the existence of an external NADH site at the cell surface responsive to the antitumor sulfonylureas.

4. Discussion

The sulfonylurea antitumor agents are unknown in their mode of action. Plasma membranes isolated from HeLa cells bind [^{3}H]LY181984 with high affinity (K_d of 20–50 nM) [1]. A binding protein was subsequently identified [12] and the isolated plasma membrane vesicles were shown to exhibit a sulfonylurea-inhibited NADH oxidase activity [2].

In this report, we provide additional evidence that the NADH oxidase of HeLa plasma membranes is sensitive to inhibition by active (LY181984) but not inactive (LY181985) experimental sulfonylurea antitumor agents. With the sulfonylurea active in inhibition of HeLa growth, this inhibition occurs at very low doses of drug. Half maximal inhibition is observed at about 30 nM.

A major anomaly of the inhibition data with LY181984 was that the drug inhibited the NADH oxidase of intact, right side-out vesicles nearly completely. However, with detergent-disrupted membranes, NADH oxidase activity was inhibited only by about 50%. With both preparations, maximal inhibition was obtained at an optimum LY181984 concentration of about 1 μM. When assays were conducted on populations of membrane vesicles that had been frozen and thawed to yield a mixture of inside-out and right side-out vesicles [13], the inhibition also was only about 50%, suggestive of a differential response of a surface exposed NADH site compared to an internal site.

With HeLa cells, the plasma membrane isolates from aqueous two-phase partition were electrophoretically homogeneous. However, after repeated freezing and thawing accompanied by passing the membrane through a narrow gauge needle [14] to evert some of the vesicles, the preparations exhibited an electrophoretic heterogeneity that appeared to result from the presence of two distinct populations of vesicles of different orientations. The existence of two plasma membrane populations were observed previously in free-flow electrophoretic separations of total cell homogenates based on measurements of the plasma membrane marker K^+-stimulated, ouabain-inhibited, *p*-nitrophenylphosphatase [15]. One population was clearly resolved as a highly electrophoretically mobile fraction of plasma membrane vesicles. The second population was of lower electrophoretic mobility. That plasma membrane vesicles of different orientations could be resolved by preparative free-flow electrophoresis was first indicated from work with erythrocyte ghosts [14].

The two populations of HeLa plasma membrane vesicles resolved by preparative free-flow electrophoresis from the purified plasma membranes prepared by aqueous two-phase partition were subsequently characterized as to content and orientation of plasma membrane markers. The population of greatest electrophoretic mobility was shown from concanavalin A binding and latency measurements to exhibit a right side-out orientation whereas the fraction of lesser electrophoretic mobility consisted of vesicles exhibiting an inside-out orientation [7].

When HeLa plasma membrane vesicles were resolved after freezing and thawing into enriched populations of right side-out and inside-out vesicles, the NADH oxidase inhibited by LY181984 was associated largely with the right side-out vesicles. Therefore, there appeared to be two NADH binding sites capable of supporting NADH oxidase activity. One appeared to be on the outside of the plasma membrane and inhibited by sulfonylurea. The other is on the inside and not inhibited by sulfonylurea. At present it is not known if these differences represent two sites on a common transmembrane complex, different isoforms of differing sulfonylurea responsiveness or completely different activities, one sulfonylurea responsive and the other sulfonylurea unresponsive.

An external NADH site for NADH oxidation by mammalian cells is supported by observations that intact cells oxidize NADH in the absence of external electron acceptors [16,17]. NADH is normally regarded as being impermeant. That this external site corresponds to the external site deduced from the vesicle sidedness studies is indicated since with HeLa and HeLa S cells grown in culture, the NADH oxidation by intact cells is sulfonylurea-responsive (L.-Y. Wu, D.M. Morré and D.J. Morré, unpublished results).

Why the plasma membrane should have an external NADH site cannot be answered at present. Since intact cells oxidize NADH directly and this NADH oxidation is sulfonylurea-inhibited, the external site appears to be both present and functional. However, cells either in culture or in tissues, would rarely have access to reduced pyridine nucleotide. A likely explanation is that the external site, while capable of oxidizing NADH, normally is involved in some function (e.g., protein disulfide-thiol interchange [18]) other than the direct oxidation of NADH.

The function of the plasma membrane NADH oxidase and especially the growth factor and/or hormone-stimulated component [19,20] is unknown. However, the NADH oxidase of plasma membranes from transformed cells and tissues fails to respond to growth factors and hormones [21,22]. Thus the oxidase, or some proteins associated with the response of the NADH oxidase to hormones and growth factors appears to be altered in transformation. Similarly, the oxidase activity of HeLa cell plasma membranes and plasma membranes of human colon adenocarcinoma [23] or ras-transformed rat kidney cells responds to the antitumor sulfonylureas [24] whereas that of rat liver and normal rat kidney cells does not. One would expect that an antitumor drug directed to a site altered in transformation might be both selective and effective. The NADH oxidase activity of the HeLa plasma membrane would represent such a site. Its apparent accessibility to NADH only with right side-out vesicles and its inhibition by an active anticancer sulfonylurea suggests that the oxidase or some associated protein may function as an extracellular target of antitumor drug action.

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