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Biochimica et Biophysica Acta 1369 (1998) 185–192

BIOCHIMICA ET BIOPHYSICA ACTA
BBA

Response of a cell-surface NADH oxidase to the antitumor sulfonylurea *N*-(4-methylphenylsulfonyl)-*N′*-(4-chlorophenylurea) (LY181984) modulated by redox

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Received 8 July 1997; accepted 13 August 1997

Abstract

In previous reports, our laboratory has described a drug-responsive NADH oxidase activity of the external surface of the plasma membrane of HeLa and other cancer cells, but not from normal cells, that was shed into media conditioned by the growth of cancer cells such as HeLa and also into sera of cancer patients. The sulfonylurea-altered activity was found in sera of a wide variety of cancer patients but the activity was either inhibited or stimulated by 1 μM LY181984. In this report, we demonstrate that one basis for whether or not the activity was stimulated or inhibited may be the redox environment of the protein. If plasma membrane vesicles from HeLa cells were first treated with dithiothreitol (DTT) or with reduced glutathione (GSH) and then assayed for NADH oxidase activity, the sulfonylurea inhibited the activity in a concentration-dependent manner. In contrast, if the plasma membrane vesicles were first treated with diluted hydrogen peroxide or oxidized glutathione (GSSG) and then assayed for NADH oxidase activity, the antitumor sulfonylurea stimulated the activity. Growth experiments were conducted in parallel. LY181984 administered to HeLa cells in the presence of GSH was approximately 2 log orders more effective than LY181984 administered to HeLa cells in the presence of GSSG. Similar results were found in the sera of cancer patients. With sera from normal individuals or with plasma membranes of rat liver, the oxidizing or reducing conditions were without effect. The findings suggest that the response of the cell surface NADH oxidase of HeLa cells to the antitumor sulfonylurea LY181984 is influenced by the redox environment which may determine whether the drug will stimulate or inhibit the activity and that the degree of response may be reflected in the ability of LY181984 to inhibit HeLa cell growth. © 1998 Elsevier Science B.V.

Keywords: Diarylsulfonylurea; Sulfonylurea; Antitumor drug; NADH oxidase; Redox; Growth; Oxidant; Glutathione; Plasma membrane; HeLa cell

1. Introduction

Plasma membrane vesicles of cultured HeLa S cells bound the tritiated antitumor sulfonylurea ([^{3}H]LY181984) *N*-(4-methylphenylsulfonyl)-*N′*-(4-chlorophenylurea) with high affinity (K_d of ca. 30 nM) [1]. Subsequently, a binding protein with one or more thiols in an active site, was identified and labeled with radioactive thiol reagents in sulfonylurea protection experiments. Binding proteins of ca. 34 kD M_r were labeled [2].

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PII S0005-2736(97)00202-2

The 34 kD sulfonylurea binding protein was demonstrated to be correlated with an NADH oxidase activity [3]. The antitumor sulfonylurea LY181984 inhibited the oxidation of NADH by isolated vesicles of plasma membranes from HeLa cells. Both right side-out and inside-out vesicles of plasma membranes from HeLa cells oxidized NADH but only the oxidation of NADH with right side-out vesicles was inhibited by LY181984 [4]. These findings suggested that the oxidation of NADH by plasma membrane vesicles from HeLa cells was inhibited by LY181984 at an external site [4]. The LY181984-inhibited NADH oxidase was subsequently shown to be released by HeLa cells and to accumulate in culture media conditioned by growth of HeLa cells. The shed form of the activity was isolated from conditioned culture media and shown to correlate with a ca. 33.5 kD protein on SDS-PAGE [5].

A shed form of the activity was found, as well, in sera from tumor-bearing rats and from cancer patients [6]. The serum form showed the same drug specificity as the plasma membrane-associated form and that shed into culture media by HeLa cells. The NADH oxidase was responsive to the antitumor sulfonylurea *N*-(4-methylphenylsulfonyl)-*N′*-(4-chlorophenylurea) (LY181984) with sera from both tumor-bearing rats and cancer patients. With sera from control rats, normal volunteers or patients with disorders other than cancer, the drug was without effect on the NADH oxidase activity of the sera. With sera from cancer patients, LY181984 added at a final concentration of 1 μM either inhibited or stimulated the activity. This was in contrast to results with HeLa plasma membrane and the culture media conditioned by growth of HeLa cells where the response to LY181984 was inhibition.

In this report, we document a response of the NADH oxidase activity of HeLa cell plasma membranes and of sera to redox environment where the same preparations will respond predominantly to the antitumor sulfonylurea either as stimulation of activity (oxidized) or as inhibition of activity (reduced). The ability of reducing or oxidizing conditions to modulate the NADH oxidase activity correlate as well with the ability of reducing or oxidizing conditions to modulate the growth of HeLa cells in response to the anticancer drug.

2. Materials and methods

2.1. Growth of cells

HeLa S cells were grown on Dulbecco's Modified Eagles Medium (D-MEM) (Joklik modified) (Gibco) with glutamine (244 mg/l) and phosphate (1.3 g/l Na_2HPO_4) and without $CaCl_2$ plus 5% donor horse serum. Gentamicin sulfate (50 μg/l) (Sigma) and sodium bicarbonate (2 g/l) were added. Cells were collected by centrifugation for 6 min at 3000 rpm.

Attached HeLa cells (ATCC CCL2), were grown in 25 cm flasks in Minimal Essential Medium (MEM) (Gibco), pH 7.4, at 37°C with 10% fetal calf serum (heat-inactivated), plus 50 μg/l gentamicin sulfate (Sigma).

2.2. Purification of plasma membranes from HeLa cells

HeLa cells grown as suspension cultures were collected by centrifugation for 6 min at $1500 \times g$. 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 10–30 min to swell the cells. Homogenization was with a Polytron homogenizer for 40 sec at 10 000 rpm using a 10 ST-probe and 7 ml aliquots. Cell survival was determined by Eosin Y exclusion. To estimate breakage, the cells were monitored by light microscopy before and after homogenization. At least 90% cell breakage without breakage of nuclei was achieved routinely.

The homogenates were centrifuged for 10 min at $175 \times g$ to remove unbroken cells and nuclei and the supernatant was centrifuged a second time for 30 min at $25\,000 \times g$ to prepare a plasma membrane-enriched microsome fraction. The supernatant was discarded and the pellets were resuspended in 0.2 M potassium phosphate buffer in a ratio of approximately 1 ml per pellet from 5×10^8 cells. The resuspended membranes were then loaded onto the two-phase system constituted on a weight basis as follows. The two-phase system contained 6.6% (w/w) Dextran T-500 (Pharmacia), 6.6% (w/w) polyethylene glycol 3350 (Fisher), and 0.2 M potassium phosphate buffer (pH 7.2) [7]. The weight of the system was brought to 14 g with distilled water.

Resuspended microsomes (2 g) were added to the two-phase system to a final weight of 16 g. The tubes were inverted vigorously for 40 times in the cold (4°C). The phases were separated by centrifugation at 750 rpm ($150 \times g$) in a Sorvall HB 4 rotor for 5 min. The upper phases were carefully withdrawn with a Pasteur pipette, divided in half and transferred into 40 ml plastic centrifuge tubes and diluted with 1 mM cold $NaHCO_3$ and centrifuged at $33\,300 \times g$ in a HB-4 rotor for 30 min. The purity of the plasma membrane was determined to be $> 90\%$ by electron microscope morphometry. The yield was 20 mg plasma membrane protein from 10^{10} cells.

2.3. Spectrophotometric assay

NADH oxidase activities of sera were determined as the disappearance of NADH measured at 340 nm with 430 nm reference using an SLM Aminco DW-2000 spectrophotometer in the dual wavelength mode of operation with continuous recording over 5 min intervals once steady-state rates were attained. The reaction mixture contained 50 mM Tris–Mes buffer (pH 7.0), 2 mM KCN to inhibit any potential mitochondrial oxidase activity, and 150 μM NADH in a total volume of 2.5 ml. Assay was at 37°C with constant stirring.

For HeLa cell plasma membranes, the spectrophotometric assay was the same except that the reaction was monitored by the decrease in absorbance at 340 nm using a Hitachi Model U3210 spectrophotometer. Changes in absorbance were recorded as a function of time by a chart recorder with both instruments. A millimolar extinction coefficient of $6.22\,cm^{-1}$ was used to determine NADH disappearance.

Proteins were determined by the bicinchoninic acid (BCA) assay [8] using bovine serum albumin as standard.

2.4. Growth measurement

Attached HeLa cells were treated in 35×10 mm plastic dishes in 2.5 ml culture medium [9]. Sulfonylurea was dissolved in DMSO and added in 2.5 μl of DMSO to yield a final DMSO concentration of 0.1%. Controls received 2.5 μl of DMSO. Growth was determined from cell numbers estimated by counting the number of cells over defined areas consisting of a grid of 1 mm squares after 24, 48 and 72 h of treatment and by means of a hemocytometer after 96 h of treatment. To release cells for counting, cells were trypsinized with 0.05% Sigma 1 X trypsin containing 0.53 mM EDTA for 1 min.

3. Results

HeLa cell plasma membrane vesicles exhibited a rate of NADH oxidation of about 1 nmoles/min/mg protein. The activity normally was inhibited by LY181984 with an LC_{50} of about 30 nM [3]. NADH oxidation was proportional to time and protein concentration. The K_m for NADH oxidation was about 25 μM [3].

The rate of NADH oxidation in the absence of sulfonylurea was only slightly altered by redox conditions (Fig. 1). With reducing conditions provided by 1 μM dithiothrietol (DTT), the activity was inhibited by LY181984 with an EC_{50} of about 30 nM (Fig. 1). However, in the presence of oxidizing conditions,

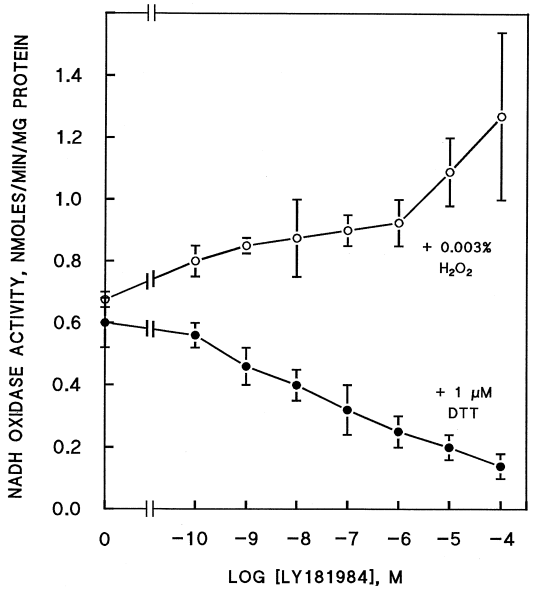

Fig. 1. NADH oxidase activity of isolated vesicles of plasma membranes from HeLa cells as a function of the concentration of the antitumor sulfonylurea, LY181984, in the presence of either 1 μM dithiothreitol or 0.003% hydrogen peroxide. Results are averages from 3 different plasma membrane preparations ± standard deviations.

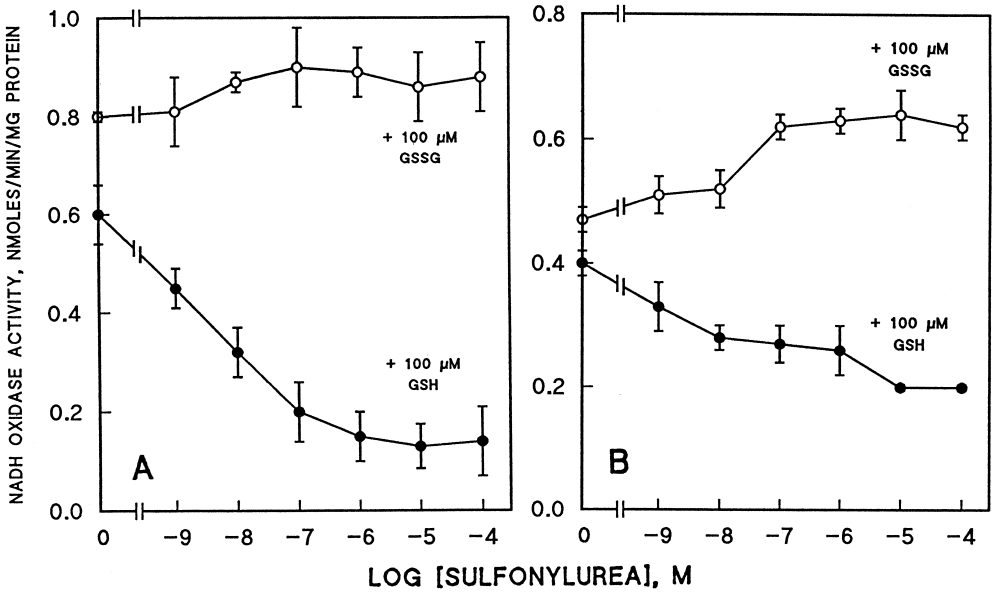

Fig. 2. NADH oxidase activity of isolated vesicles of plasma membranes from HeLa cells as a function of the concentration of LY181984 in the presence of either 100 μM reduced (GSH) or oxidized glutathione (GSSG). (A) Freshly frozen plasma membranes. (B) Plasma membranes stored frozen for 6 months. Results are averages from 4 different plasma membrane preparations ± standard deviations.

i.e., 0.003% hydrogen peroxide, the LY181984 no longer was inhibitory. Rather, stimulations were observed (Fig. 1).

When the response to varying concentrations of LY181984 was measured in the presence of either reduced (GSH) or oxidized (GSSG) glutathione, inhibition by LY181984 was enhanced with GSH. With GSSG, not only did the LY181984 fail to inhibit NADH oxidation, but a slight stimulation was observed (Fig. 2(A)). When assayed with preparations of lower starting specific activity (stored longer), the degree of inhibition with GSH also was less (Fig. 2(B)). However, GSSG under these conditions stimulated activity over the entire range of GSSG concentrations tested.

In contrast to HeLa plasma membranes, the shed form of the drug-responsive NADH oxidase was either stimulated or inhibited by LY181984 depend-

Table 1
Response of NADH oxidase of patient sera (−SU) and response to 1 μM LY181984 in DMSO (+SU) comparing oxidizing (0.003% H_2O_2) or reducing (1 μM DTT) conditions. Units are NADH oxidase, nmoles/ml serum

Cancer	Designation	No Addition			0.003% H_2O_2			1 μM DTT		
		− SU	+ SU	Ratio	− SU	+ SU	Ratio	− SU	+ SU	Ratio
Breast	SB-6	1.1	0.55	0.5	0.6	0.7	1.17	0.2	0.12	0.6
	SB-21	0.22	0.35	1.6	0.9	1.2	1.33	0.55	0.4	0.55
	SB-22	0.3	0.2	0.7	1.3	1.2	0.92	0.7	0.6	0.8
	SB-104	0.45	0.35	0.8	1.5	1.7	1.13	0.6	0.5	0.8
Prostate	SB-4	0.4	0.9	2.25	0.8	1.0	1.25	0.6	0.4	0.7
Ovarian	SB-33	0.5	0.35	0.7	0.6	0.65	1.08	0.35	0.15	0.4
Leukemia (CLL)	SB-29	0.5	0.3	0.6	0.45	1.0	2.22	0.2	0.1	0.5
Pancreatic	SB-9	0.5	0.8	1.6	0.68	0.83	1.22	0.65	0.55	0.85
Bladder	SB-69	0.4	0.35	0.9	0.35	0.5	1.43	0.3	0.1	0.3
Lung	SB-5	0.4	0.2	0.5	0.9	1.1	1.22	0.5	0.25	0.5

Table 2
Summary of NADH oxidase activity of patient sera (−SU), response to 1 μM LY181984 in DMSO (+SU) and modulation by oxidizing or reducing conditions. Units of specific activity are NADH oxidase, nmoles/ml serum

Cancer	Number of Patients	Redox Addition	−SU	+SU	Ratio	0.5–0.85	0.9–0.95	1.0	1.05–1.1	1.15–2.0
Breast	8	No addition	0.66 ± 0.2	0.62 ± 0.2	0.94 ± 0.3	4	0	1	0	3
	8	0.003% H_2O_2	0.85 ± 0.4	0.99 ± 0.4	1.197 ± 0.06	0	1	0	0	7
	7	1 μM DTT	0.81 ± 0.53	0.63 ± 0.38	6.71 ± 0.29	6	0	0	0	1
Leukemia	9	No addition	0.39 ± 0.15	0.40 ± 0.1	1.06 ± 0.45	4	1	0	0	4
+Lymphoma	9	0.003% H_2O_2	0.58 ± 0.3	0.65 ± 0.3	1.26 ± 0.5	2	0	0	0	7
	6	1 μM DTT	0.68 ± 0.5	0.4 ± 0.3	0.6 ± 0.2	6	0	0	0	0
Lung	5	No addition	0.6 ± 0.2	0.6 ± 0.2	0.94 ± 0.3	3	0	0	0	2
	5	0.003% H_2O_2	0.9 ± 0.3	1.07 ± 0.3	1.19 ± 0.06	0	0	0	0	5
	5	1 μM DTT	0.5 ± 0.1	0.25 ± 0.05	0.6 ± 0.02	4	0	0	0	1
Colon	5	No addition	0.6 ± 0.2	0.5 ± 0.1	0.95 ± 0.2	3	0	0	0	2
	5	0.003% H_2O_2	0.6 ± 0.2	0.8 ± 0.2	1.26 ± 0.13	0	0	0	0	5
	5	1 μM DTT	0.5 ± 0.2	0.35 ± 0.15	0.7 ± 0.1	5	0	0	0	0

ing on the particular sample (Table 1, Ref. [6]). Of 201 serum samples examined previously, 55% were inhibited by sulfonylurea (either LY181984 or LY217447) and 44% were stimulated by sulfonylurea (LY181984).

To determine if the serum form also responded to redox, sera were tested in the presence of 0.003% hydrogen peroxide or 1 μM DTT (Table 1). In general, initial rates were somewhat greater with hydrogen peroxide than with DTT. However, a most marked change in the response to sulfonylurea was seen with redox environment. For the 27 samples of cancer patient sera of Table 2, the initial response to 1 μM LY181984 was a nearly equal mixture of stimulations and inhibitions. However, with 0.003% hydrogen peroxide, the dominant response to 1 μM LY181984 was stimulation whereas with 1 μM DTT the dominant response to LY181984 was inhibition.

The differences were not absolute however. Of the four breast cancer sera of Table 1, one was not stimulated by 1 μM LY181984 in the presence of 0.003% hydrogen peroxide. With the data summarized in Table 2, of the 27 samples of sera from cancer patients, 1 μM LY181984 inhibited in 15 of the samples and stimulated with 11 in the absence of any additions. With 0.003% hydrogen peroxide, 1 μM LY181984 stimulated in 24 of the 27 sera tested and with 1 μM DTT, 1 μM LY181984 inhibited with 21 of the 23 samples tested. The same set of serum samples was represented in each of the different series so that there was a clear shift away from a random distribution of stimulations and inhibitions by 1 μM LY181984 to inhibition under reducing conditions and stimulation under oxidizing conditions. The one specimen of sera from a breast cancer patient neither inhibited nor stimulated by LY181984 with no addition was stimulated by LY181984 (+SU/−SU ratio of 1.28) in the presence of 0.003% hydrogen peroxide. Results with 0.003% and 0.03% hydrogen peroxide were equivalent.

Table 3
NADH oxidase activity of sera from healthy laboratory volunteers (−SU) and response to 1 μM antitumor sulfonylurea, LY181984 in DMSO (+SU) comparing different redox conditions during assay

Redox Condition	N	NADH oxidase nmoles/ml serum		Ratio, +SU/−SU
		−SU	+SU	
No Addition	25	0.38 ± 0.08	0.38 ± 0.08	1.0 ± 0.02
0.003% H_2O_2	23	0.78 ± 0.29	0.77 ± 0.28	0.98 ± 0.06
0.03% H_2O_2	10	0.74 ± 0.21	0.74 ± 0.21	1.0 ± 0.01
1 μM DTT	15	0.6 ± 0.3	0.6 ± 0.3	1.0 ± 0.03
10 μM DTT	4	0.3 ± 0.1	0.3 ± 0.1	1.0 ± 0.05

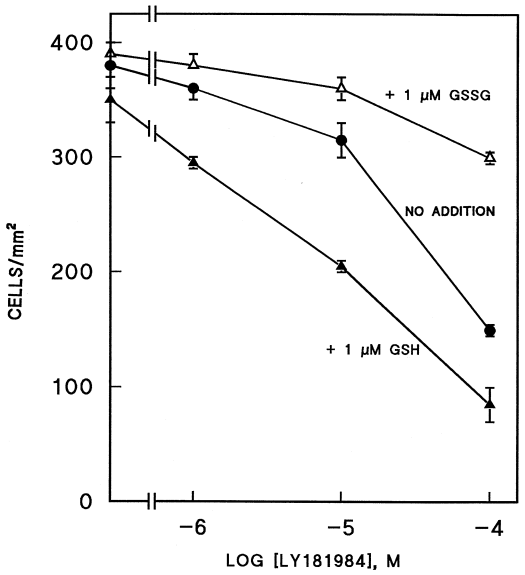

Fig. 3. Growth of HeLa cells after 96 h as a function of the concentration of LY181984. The solid circles are with no addition. The open triangles are with 1 μM GSSG and the solid triangles are with 1 μM GSH. These concentrations of GSH and GSSG were selected to be within the range of concentrations not affecting the growth of HeLa cells in the absence of LY181984. Values are from three determinations ± standard deviations.

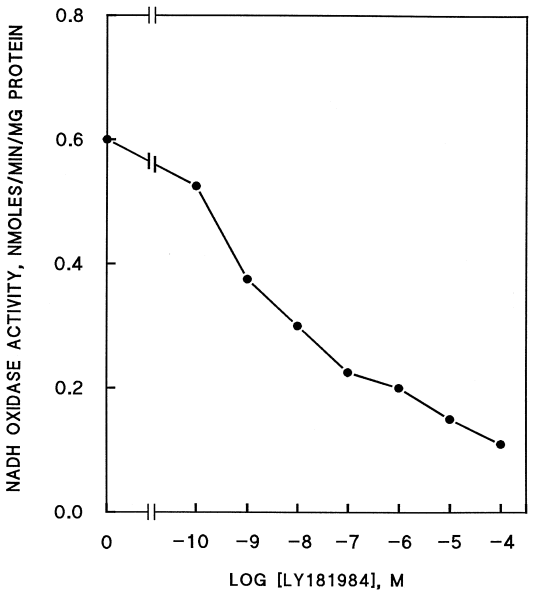

Fig. 4. NADH oxidase activity of plasma membrane vesicles isolated from HeLa cells and assayed in the presence of varying concentrations of LY181984 in the presence of 100 μM L-cysteine.

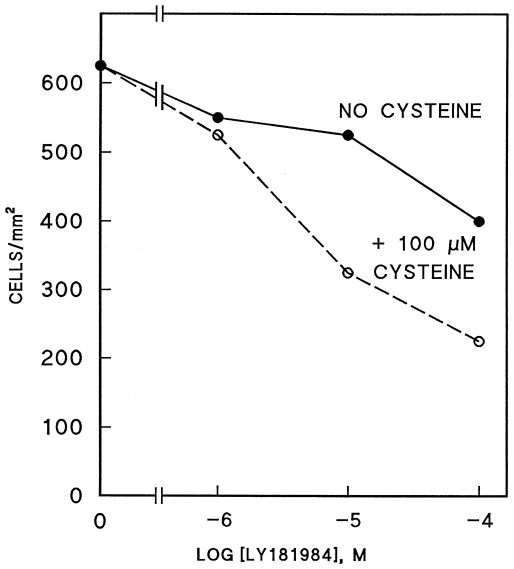

Fig. 5. Growth of HeLa cells after 96 h as a function of sulfonylurea concentration in the presence or absence of 100 μM L-cysteine.

With sera from healthy volunteers, the hydrogen peroxide appeared to stimulate activity although the increase was not highly significant (Table 3). However, none of the oxidizing or reducing conditions resulted in a response, either stimulation or inhibition, to 1 μM of the sulfonylurea LY181984 of more than ±6% (Table 3).

Not only did the redox environment affect the response of the NADH oxidase to LY181984, it also altered the growth response of HeLa cells to LY181984. Reduced glutathione at a concentration of 1μM increased the efficacy of LY181984 by about one log order in magnitude compared to no addition (Fig. 3). With 1 μM GSSG, the efficacy of LY181984 in growth inhibition was decreased by at least one log order compared to no addition (Fig. 3).

The inhibition of NADH oxidation by LY181984 was enhanced by cysteine (Fig. 4). When cysteine was added to the culture medium, the ability of LY181984 to kill HeLa cells also was enhanced (Fig. 5).

4. Discussion

The activity of cell surface NADH oxidase correlates with the growth rate of HeLa cells [6]. When

inhibited, as with capsaicin [10] or by the antitumor sulfonylurea [11], or with submicromolar concentrations of retinoic acid [12], the cells appear to undergo apoptotic cell death as evidenced by an increase in DAPI fluorescence [10,11] and DNA fragmentation [12]. At much higher concentrations (e.g. 100 μM), capsaicin and retinoic acid also slowed growth and induced apoptosis in non-transformed cells [9,10]. Wolvetang et al. [13] demonstrated that the NADH oxidase-mediated apoptosis was blocked by antisera to the cell surface oxidase and could be reversed by addition of coenzyme Q. The response of transformed cells to these apoptosis-inducing agents is much more specific than with nontransformed cells. Inhibitions occurred with transformed cells in the submicromolar and nanomolar range. These concentrations have no effect either on the NADH oxidase or on growth or survival of non-transformed cells [10].

The drug-responsive NADH oxidase is assumed to be widely distributed among different tumor types. This supposition is based on the observation that a sulfonylurea-responsive NADH oxidase is present in sera of patients with late-stage cancer representing a variety of solid tumors, leukemias and lymphomas [6]. The activity has also been demonstrated directly with a small number of human tumors and transformed cell lines (HeLa, MCF10A mammary adenocarcinoma, HL-60 leukemia, colon xenografts, glioblastoma). Sera from normal volunteers [6] and nontransformed cells and tissues [14] appeared to lack the activity.

A major anomaly encountered with the sera samples was that, while the majority did respond to sulfonylurea, the responses were nearly equally divided among inhibition and stimulation [6]. The variations were reproducible and not correlated with tumor type, gender, age, conditions of sera collection or storage or geographic distribution or therapy. The possibility that the response might be related to the oxidation or reduction state of the oxidase was first raised with binding experiments where binding of radiolabeled LY181984 to isolated plasma membrane vesicles of HeLa was enhanced in the presence of reduced glutathione or dithiothreitol and reduced with hydrogen peroxide [2].

When effects of similar treatments were evaluated with NADH oxidase activities of HeLa cell plasma membranes, a very marked effect on the sulfonylurea response was observed. As with sulfonylurea binding, if the membranes were in a reducing environment and sulfonylurea binding affinity was enhanced, so was the degree of inhibition of activity enhanced. This response was seen also with sera of cancer patients where, with no addition, approximately half responded to LY181984 by inhibition of activity. The majority of the remainder were stimulated. However, in the presence of DTT, LY181984 resulted in inhibition in $>90\%$ of the serum samples including samples where, in the absence of DTT, the LY181984 resulted in a stimulation of activity. In an oxidizing environment, the NADH oxidase activity of the plasma membrane vesicles was stimulated.

Activity responses of enzymatic activities [15] and receptor function [16,17] to redox conditions are not without precedent. The usual interpretation involves some dependence on the breakage or formation of disulfide bonds [15]. With the effect on the NADH oxidase, the response of the membrane-associated forms would appear to be the result of a direct effect on the protein itself since an altered response to drug was seen as well with the shed form of the activity in sera of cancer patients.

An unexpected outcome of an explanation based on affinity of drug binding was the stimulation of NADH oxidation in response to LY181984 under oxidizing conditions imposed by GSSG or dilute hydrogen peroxide. While it may be that oxidizing conditions in serum, for example, favor a stimulatory drug response, other factors also may be involved since the response of serum samples to the redox environment was not absolute. What seems to occur and was seen also with the data for HeLa cells of Fig. 2, was that oxidation tended to reduce inhibition whereas reduction tended to enhance inhibition against some background level of drug response that may also influence degree of drug inhibition or stimulation of the enzymatic activity, i.e., different activity forms.

The response to oxidizing and reducing conditions does not appear to be that of a cosubstrate. For example, with the response to dithiothreitol, there was no clear dose dependency. The response was approximately the same at 1, 10 and 100 μM. The response to hydrogen peroxide was similar with 0.0003, 0.003 and 0.03%. Also results with dithiothreitol, reduced glutathione and cysteine were equiv-

alent as were results with oxidized glutathione and hydrogen peroxide. A direct effect on the drug was ruled out for dithiothrietol and glutathione, both oxidized and reduced, using the radiolabeled drug (1, 2).

Despite the possibility of yet unappreciated levels of complexity, the redox state of the NADH oxidase appears to have relevance in terms of the growth response of HeLa cells to the sulfonylurea. The enhancement of inhibition of the oxidase and enhanced binding to plasma membranes [1,2] is reflected in an enhanced inhibition of growth under reducing conditions achieved either with reduced glutathione or dithiothreitol. With the oxidizing conditions afforded by oxidized glutathione, the sulfonylurea was nearly without effect on the growth of cells. These findings suggest the possibility of therapeutic benefit from modifying the redox environment of anticancer drugs which may target the cell surface NADH oxidase. A response to oxidation or reduction was observed with mouse melanoma and the vanilloid, capsaicin (8-methyl-*N*-vanillyl-6-noneamide), both in vitro and in vivo with tumors transplanted subcutaneously [18].

References

[1] D.J. Morré, D.M. Morré, J. Stevenson, W. MacKellar, D. McClure, Biochim. Biophys. Acta 1244 (1994) 133–140.
[2] D.J. Morré, F.E. Wilkinson, J. Lawrence, N. Cho, M. Paulik, Biochim. Biophys. Acta 1236 (1995) 237–243.
[3] D.J. Morré, L.-Y. Wu, D.M. Morré, Biochim. Biophys. Acta 1236 (1995) 237–243.
[4] D.J. Morré, Biochim. Biophys. Acta 1240 (1995) 201–208.
[5] D.J. Morré, F.E. Wilkinson, C. Kim, N. Cho, J.L. Lawrence, D.M. Morré, D. McClure, Biochim. Biophys. Acta 1236 (1996) 237–243.
[6] D.J. Morré, T.J. Reust, Bioenerg. Biomemb. 29 (1997) 283–291.
[7] D.J. Morré, D.M. Morré, Biotechnology 7 (1989) 946–958.
[8] P.K. Smith, R.I. Krohn, G.T. Hermanson, A.K. Mallia, F.H. Gartner, E.K. Provenzano, E.K. Fujimoto, N.M. Goeke, B.J. Olson, D.C. Klenk, Annal. Biochem. 100 (1985) 76–85.
[9] S. Dai, D.J. Morré, C.C. Geilin, B. Almond-Roesler, C.E. Orfanos, D.M. Morré, Mol. Cell. Biochem. 166 (1997) 101–109.
[10] D.J. Morré, P.-J. Chueh, D.M. Morré, Proc. Natl. Acad. Sci. U.S.A. 92 (1995) 1831–1835.
[11] D.M. Morré, D.J. Morré, Protoplasma 184 (1995) 188–195.
[12] D.M. Morré, S. Dai, L.- Y. Wu, D.J. Morré, FASEB J. 10 (1996) A525.
[13] E.J. Wolvetang, J.A. Larm, P. Moutsoulas, A. Lawen, Cell Growth Differ. 7 (1996) 1315–1325.
[14] D.J. Morré, R. Merriman, L.R. Tanzer, L.-Y. Wu, D.M. Morré, W.C. MacKellar, Protoplasma 184 (1995) 203–208.
[15] H.F. Gilbert, J. Biol. Chem. 254 (1982) 12086–12091.
[16] D.I. Levy, N.J. Sucher, S.A. Lipton, Neurosci. Lett. 110 (1990) 291–296.
[17] M.D. Majewska, J.A. Bell, E.O. London, Brain Res. 537 (1990) 328–332.
[18] D.J. Morré, C. Geilin, L.-Y. Wu, R. deCabo, K. Krasagakis, C.E. Orfanos, D.M. Morré, Eur. J. Cancer 32A (1996) 1995–2003.