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Oxalate-Induced and Cell-Cycle-Dependent Expression of Nuclear Pore Complex Oxalate Binding Protein gp210

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The effect of oxalate, a constituent of renal stone, on the expression of nuclear pore complex oxalate binding protein (gp210) in Vero monkey kidney cells was examined. The expression of this protein was found to increase more in mitotic phase than in S phase, suggesting cell cycle dependency. Exposure of cells to oxalate-containing growth medium resulted in a relative increase in nuclear pore complex oxalate binding protein in each stage of cell cycle. The concentration of this protein was found to increase six times in the telophase stage of the cells exposed to high concentrations of oxalate in the growth medium, though slight reduction in cell density was observed. Structural analogues of oxalate did not show any stimulatory effect on expression of this oxalate binding protein. Hence, the expression of the nuclear pore complex oxalate binding protein gp210 was specific to oxalate and is cell cycle dependent.

Key Words: **oxalate binding protein, nuclear pore complex; gp210; cell cycle.**

Urolithiasis is a process of biomineralixation in the urinary tract. Perturbations in renal oxalate handling play a major role in renal stone disease (1). Our earlier studies have shown the presence of about 70% of total oxalate binding to nuclei while only 30% binding to the mitochondria (2,3). Our earlier studies have reported the presence of oxalate binding protein in the nuclear envelope (4). Our preliminary studies have shown that the nuclear pore complex protein gp210 is an oxalate binding protein (5). Oxalate has been shown to induce mitosis (6), DNA synthesis (7), and expression of certain genes (8). The physiological significance of the presence of oxalate binding protein in the nuclear pore complex is not well understood. In order to study its functions, the expression of this protein during different stages of cell cycle was studied in the presence of oxalate.

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MATERIALS AND METHODS

Minimum essential medium, penicillin, streptomycin, sodium bicarbonate, fetal calfserum, methotrexate, colchicin, Cytochalasin B, trypsin-EDTA solution, and molecular weight markers were obtained from Sigma Chemical Company, St. Louis, Missouri. Sodium oxalate, oxamate, malate, citrate. and other chemicals were purchased from Sisco Research Laboratories, Mumbai. Other chemicals used were of analytical grade and purity.

Human primary biliary cirrhosis (PBC) serum containing autoantibodies against nuclear pore complex protein gp210 was a gift from Professor Howard J. Worman, Columbia University, New York (9). Goat anti-human IgG horseradish peroxidase was a gift from NII, New Delhi.

Cell *line and cell culture.* Vero monkey kidney cells were serially passaged in minimal essential medium (MEM) supplemented with 10% fetal calf serum, penicillin (100 U/ml), and streptomycin (100 µg/ml). The cells were maintained in an atmosphere of 5% CO_2/95% air in a humidified 37°C incubator.

Oxalate concentration. Oxalate concentration in the medium was fixed according to (10) to be 0.1 mM (low), 0.3 mM (medium), and 1 mM (high/toxic) total oxalate (which is equivalent to 30, 100, and 350 µM free oxalate concentrations, respectively). 1 x 10^5 cells were incubated with MEM containing specified concentrations of oxalate.

Synchronization at different stages of cell cycle. 1 x 10^5 cells were incubated with MEM containing corresponding cell cycle blockers in 25-ml culture flasks. Cells were synchronized at S phase using 10 µg/ml methotrexate, at mitotic phase (metaphase) using 10 µg/ml colchicin, and at mitotic phase (telophase) using 10 µg/ml Cytochalasin B and the cells were allowed to synchronize for 24 h.

Substrate analogues. 1 x 10^5 cells were incubated with MEM containing 0.1 mM oxalate, malate, succinate and citrate and grown for 24 h.

Trypsinization. Cells were trypsinized by incubating with trypsin-EDTA solution for 20 minutes, and washed two times by centrifuging at 2500 rpm/10 minutes with MEM and resuspended with the same.

Assessment of cell *density.* From the resuspended pellet, an aliquot of the cell was mixed with 0.05% trypan blue. Cells were then examined immediately under a light microscope and the relative abundance of the dead (stained) cells was assessed.

Whole cell extract. Whole cell extract was prepared according to the method of (11). The cells resuspended in MEM was centrifuged at 2500 rpm/5 minutes end resuspended in the lysis buffer (100 mM Tris-HCl, pH 7.4, containing 1% Triton X-100, 0.4% SDS and 2 mM EDTA) and incubated for 30 minutes at 4°C. The Triton X-100 extract containing the nuclear pore complex protein was obtained by centrifuging the whole cell extract at 15,000 rpm/30 minutes. Protein

0006-291X/99 $30.00

TABLE 1

Effect of Oxalate on Cell Density

Particulars	% of cell density
0.1 mM Oxalate	+25
0.3 mM Oxalate	**+40**
1.0 mM Oxalate	-10

Note. +, Increase; -, decrease. Values are means of 3 experiments with 2 replicates in each experiment. 1 **x** 10^5 cells were incubated with growth medium containing varying concentrations of oxalate. After 24 h, 10 µl of cells from each category was mixed with 0.05% trypan blue and the cell density was assessed as described under Materials and Methods.

concentration was estimated by the method of (12) in the Triton X-100 extract and in the residual pellet.

ELISA. Enzyme-linked immunosorbent assay was done according to the method of (13). 30 µg of Triton extract and residual pellet were coated on 96-well microtitre plates, probed with 1:500 diluted PBC serum containing autoantibodies against nuclear pore complex protein gp210, and then incubated with 1:20,000 diluted secondary antibody (goat anti-human IgG horseradish peroxidase). TMB/H_2O_2 was added and incubated in dark. The color developed on adding 1 N H_2SO_4 was read at 540 nm on a MR600 Dynatech ELISA reader.

The total gp210 concentration (µg/1 **x** 10^5) was calculated from *a* standard curve obtained by coating varying concentrations of isolated human kidney gp210 (1-10 µg) on 96-well microtitre ELISA plates and probing with PBC serum (1:500) containing gp210 autoantibodies and proceeded as for ELISA.

SDS-PAGE. Triton extracts of unsynchronized and synchronized cells in the presence of 0.1, 0.3, and 1.0 mM oxalate were electrophoresed on an 8% mini-gel along with molecular weight markers ranging from 21 to 205 kDa (14) and silver stained by the method of (15).

Statistical analysis. All biochemical values were expressed as means ± SD of 3 experiments with 2 replicates in each experiment. The *P* significance was arrived at using Student's *t* test *(P* < 0.05*; *P* < 0.01**; *P* < 0.001***).

RESULTS

Effect of Oxalate on Cell Density

Exposure of VERO cells to oxalate in the culture medium produced changes in cell density. 24 h of exposure to varying concentrations (0.1, 0.3, and 1.0 mM total oxalate) of oxalate produced biphasic effects on cell numbers. Cells exposed to 0.1 mM total oxalate showed 25% increase in cell density, cells exposed to 0.3 mM total oxalate showed 40% increase in cell density, and those exposed to 1.0 mM total oxalate showed a slight decline of 10% in cell density compared to that of the control cells unexposed to oxalate (Table 1).

Effect of Oxalate on the Expression of gp210

When the Triton extract of cells grown in 0.1, 0.3, and 1.0 mM total oxalate were quantitated for gp210 concentration by ELISA, there was a significant increase of 63% (P < 0.01), 155% (P < 0.01), and 450% (*P* < 0.001), respectively (Fig. 1). The residual pellet showed no presence of gp210.

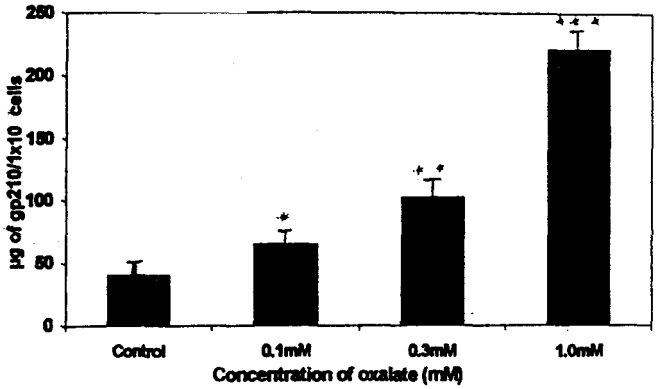

FIG. 1. Effect of oxalate on expression of gp210. Values are means ± SD of 3 experiments with 2 replicates for each experiment. 30 µg of Triton extract was coated onto ELISA plates and probed with PBC serum containing gp210 autoantibodies (1:500) and the gp210 concentration was determined as described under Materials and Methods. Values are statistically significant compared to control *(P* < 0.05*; *P* < 0.01**; *P* < 0.001***).

Oxalate Induced and Cell-Cycle-Dependent Expression of gp210

By ELISA. The concentration of gp210 in synchronized cells grown in the medium containing different concentrations of oxalate showed an increase compared to that of the control cells unexposed to oxalate (Fig. 2).

In the S phase, cells exposed to 0.1, 0.3, and 1.0 mM total oxalate showed a significant increase of 83% *(P* < 0.05), 152% *(P* < 0.01), and 213% *(P* < 0.001) of gp210 concentration respectively compared to that of the control S phase cells unexposed to oxalate.

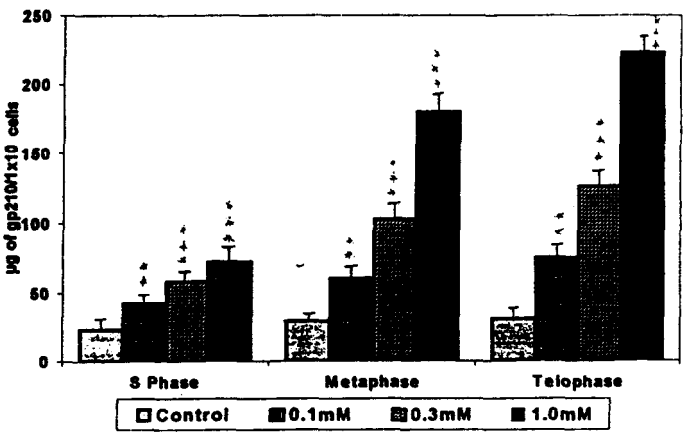

FIG. 2. Oxylate-induced and cell-cycle-dependent expression of gp210. Values are means ± SD of 3 experiments with 2 replicates for each experiment. 30 µg of Triton extract was coated onto ELISA plates and probed with PBC serum containing gp210 autoantibodies (1:500) and the gp210 concentration was determined as described under Materials and Methods. Values are statistically significant compared to control *(P* < 0.05*; *P* < 0.01**; *P* < 0.001***).

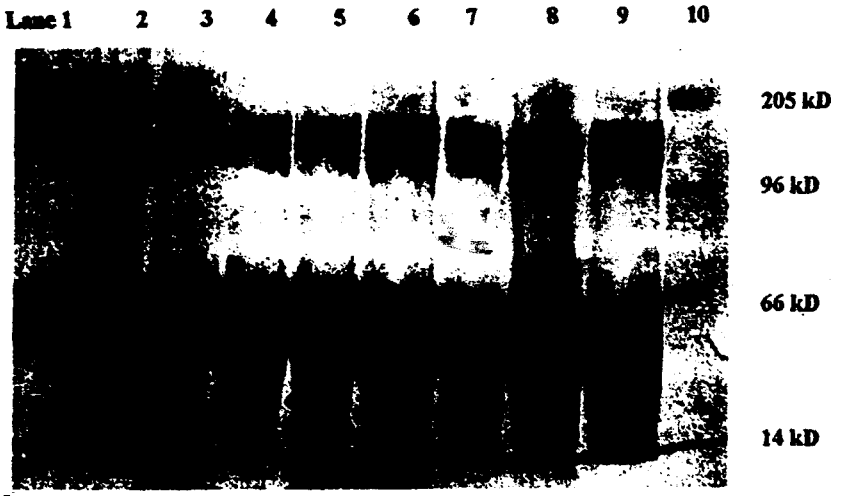

FIG. 3. SDS-PAGE profile of oxalate induced and cell cycle-dependent expression of gp210. Lane 1: S phase cells exposed to 0.1 mM oxalate. Lane 2: S phase cells exposed to 0.3 mM oxalate. Lane 3: S phase cells exposed to 1.0 mM oxalate. Lane 4: Metaphase cells exposed to 0.1 mM oxalate. Lane 5: Metaphase cells exposed to 0.3 mM oxalate. Lane 6: Metaphase cells exposed to 1.0 mM oxalate. Lane 7: Telophase cells exposed to 0.1 mM oxalate. Lane 8: Telophase cells exposed to 0.3 mM oxalate. Lane 9: Telophase cells exposed to 1.0 mM oxalate. Lane 10: Molecular weight markers. 50 μg of Triton extract of cells was electrophoresed on 8% mini-gel along with molecular weight markers and silver stained as described under Materials and Methods.

In the mitotic phase, cells exposed to 0.1, 0.3, and 1.0 mM total oxalate and synchronized at metaphase showed a significant increase of 107% ($P < 0.01$), 252% ($P < 0.01$), and 520% ($P < 0.001$) of gp210 concentration respectively compared to that of the control metaphase cells unexposed to oxalate. The cells synchronized at telophase and exposed to 0.1, 0.3, and 1.0 mM total oxalate concentration showed a significant increase of 142% ($P < 0.001$), 306% ($P < 0.001$), and 620% ($P < 0.001$) of gp210 concentration compared to that of the control telophase cells unexposed to oxalate. Telophase cells exposed to 1.0 mM total oxalate concentration showed the maximum concentration of gp210.

SDS-PAGE. The Triton extracts of synchronized cells grown in different concentrations of oxalate were electrophoresed on 8% SDS-PAGE along with molecular weight markers. Relatively thicker bands were observed in telophase cells with increase in oxalate concentration (Fig. 3).

Effect of Substrate Analogues

When cells grown with 0.1 mM oxalate or its structural analogues—oxamate, malate, succinate, and citrate-were checked for gp210 concentration, only cells grown with oxalate showed a significant increase of 63% ($P < 0.01$) of gp210 concentration, while others did not show any significant increase in gp210 concentration (Fig. 4).

DISCUSSION

In our earlier studies, we have shown that nuclear oxalate binding activity is associated with the nuclear pore complex protein gp210. The physiological importance of this activity with gp210 is not clear. Oxalate has been shown to induce mitosis (6) while gp210 is also reported to be involved in cell cycle (16). In order to understand the role of gp210 during cell cycle, the expression of this oxalate binding protein during oxalate stress condition has been determined.

Lower concentrations of oxalate increase the cell density while at higher concentration, a slight decline in cell density is observed. Available evidence suggests that oxalate levels are about 5-10 μM in the glomerular filtrate (17) and would increase to 50-100 μM in the proximal tubules. Cortical oxalate levels might reach approximately 300 μM free oxalate concentra-

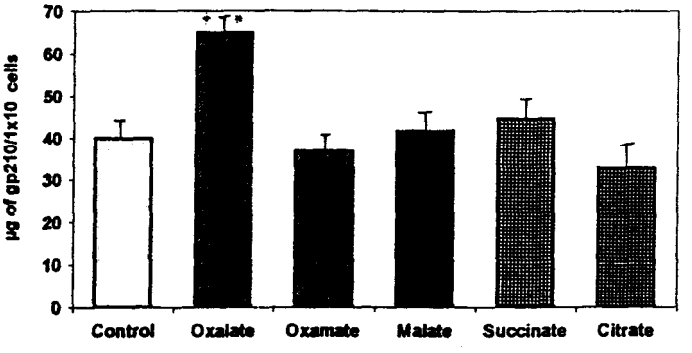

FIG. 4. Effect of substrate analogues on expression of gp210. Values are means ± SD of 3 experiments with 2 replicates for each experiment. 30 μg of Triton extract was coated onto ELISA plates and probed with PBC serum containing gp210 autoantibodies (1:500) and the gp210 concentration was determined as described under Materials and Methods. Values are statistically significant compared to control ($P < 0.01$**).

tion (18). Thus, oxalate might well approach toxic levels in the proximal tubules, particularly in individuals with hyperoxaluria. High concentrations of oxalate (1.0 mM total oxalate) are toxic to LLC-PKl cells, a line of renal epithelial cells (10). However, in our study, the cells exposed to higher concentration of oxalate (1.0 mM total oxalate) have shown an increase in gp210 concentration suggesting an induction of its expression by oxalate. Further its expression is specific only for oxalate since its structural analogues show no induction of gp210 expression.

Though the expression of gp210 is increased at each stage of the cell cycle on exposure to different concentrations of oxalate, the maximal expression of gp210 is found to be in mitosis (6-fold) rather than in interphase (2-fold). Gp210 is involved in targeting the pore proteins (19) to facilitate the formation of the pore complex (20) and also anchors (21) the pore complex during telophase. Many of the structural modifications of the cellular architecture that take place in mitosis, including nuclear envelope breakdown, occur concurrently with protein phosphorylation (22, 23). As gp210 is expressed more in mitosis during oxalate stress condition, it is suggested that oxalate plays a significant role in targeting gp210 towards reassembly of nuclear envelope for the new daughter cells. As oxalate has been shown to have mitogenic effect (6), the cell proliferation may be associated with the induction of gp210. In addition, oxalate is shown to induce the expression of *c-myc* gene (8), and calcium oxalate crystals have been shown to induce the expression of immediate early genes *c-myc, c-jun, EGR-1* and *NVR-77* and genes encoding plasminogen activator (PAI-1) (24) and platelet-derived growth factor (PDGF)-A in BAC-1 kidney epithelial cells in primary cultures of rat proximal tubular epithelium on exposure to oxidative stress (25).

Under oxalate stress conditions, the intracellular oxalate concentration is very high (26) and nuclei contain 2/3 of total oxalate (2). The presence of an oxalate binding protein in the pore complex may be very significant for the accumulation of oxalate in nuclei. Further, oxalate is shown to bind histone HlB and has been suggested to be involved with transcription (27). Hence, it is concluded that oxalate induces the expression of the nuclear pore complex oxalate binding protein gp210 in cell cycle processes.

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