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INVOLVEMENT OF CELLULAR SULFHYDRYL COMPOUNDS IN THE INHIBITION OF RNA SYNTHESIS BY SELENITE

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Abstract—Selenite has been shown previously to inhibit cellular RNA synthesis. Based upon our previous observation that selenite inhibits purified RNA polymerase only in the presence of a sulfhydryl compound (Frenkel *et al.*, *Mol Pharmacol* **31**: 112–116, 1987), we hypothesized that the inhibition of cellular RNA synthesis by selenite involves endogenous sulfhydryl compounds. We found that depletion of cells of endogenous sulfhydryl compounds, by exposure to diethylmaleate (DEM), virtually eliminated the inhibitory effect of a 1-hr exposure of cells to selenite. This inhibition was restored to normal or higher levels when the selenite was reacted with glutathione or cysteamine prior to addition to the DEM-treated cells. RNA synthesis in DEM-treated cells was inhibited after a 4-hr exposure to higher concentrations of selenite. In contrast to the effect of DEM, specific depletion of the cells of glutathione, by exposure to buthionine sulfoximine, had no effect on the inhibition of RNA synthesis by selenite. These results demonstrate the involvement of endogenous cellular sulfhydryl compounds in the inhibition of RNA synthesis by selenite, but indicate that glutathione, in particular, is not involved in this inhibition.

During the past few years, there have been a number of studies which have suggested that sulfhydryl (SH) compounds may play a role in the cellular effects of selenite [1–8]. In particular, SH compounds have been shown to potentiate a number of the toxic effects of selenite on cells, including inhibition of cell growth [5, 7] and protein synthesis [6]. In addition, selenite is known to react with SH compounds to form selenotrisulfides [9] which have been shown to produce some of the same effects on cells as selenite, sometimes with greater potency [3]. These findings are suggestive of the involvement of cellular SH compounds in the inhibitory effects of selenite, but do not provide direct evidence for it.

Cellular DNA and RNA syntheses have also been shown to be inhibited by selenite [10–12]. We recently reported that DNA and RNA polymerases, the enzymes responsible for this synthesis, are only inhibited by selenite in the presence of an SH compound [13]. This has led us to hypothesize that the inhibition of cellular nucleic acid synthesis by selenite involves endogenous cellular SH compounds. To obtain evidence for this hypothesis, we examined the effect of the depletion of cells of endogenous SH compounds on the inhibition of nucleic acid synthesis by selenite. In this paper we report the results of our studies on RNA synthesis; our studies on DNA synthesis have been reported elsewhere [14].

MATERIALS AND METHODS

Materials. Diethylmaleate (DEM), buthionine sulfoximine (BSO), glutathione, glutathione disulfide and cysteamine were purchased from Sigma Chemical Co. (St Louis, MO), and cysteamine disulfide (cystamine) was obtained from Aldrich Chemical Co. (Milwaukee, WI). [^{3}H]Uridine was purchased from New England Nuclear (Boston, MA) and sodium selenite from Gallard-Schlesinger (Carle Place, NY).

RNA synthesis in cells. HeLa cells were grown in monolayer cultures in Dulbecco's Modified Eagle's Medium (Gibco, Grand Island, NY) containing 10% fetal calf serum (Gibco). Approximately 5×10^5 cells were seeded in 60 mm dishes; 3 days after seeding, when the cultures just reached confluence, sodium selenite was added to the cultures at the concentrations indicated in the individual experiments (in most cases, 10, 20, 50, 100 and 200 μM) and incubation was continued for 1 hr at 37°. [^{3}H]Uridine (50 Ci/mmol; final concentration 3 μCi/ml) was then added and incubation continued for 15 min at 37°. The incorporation of radioactivity into acid precipitable form was then measured as described previously [15]. The results are presented as the means of the values obtained with duplicate culture dishes (which varied less than 10%). In DEM-treated cells, DEM was added to the culture (at a final concentration of 1 mM) 2 hr prior to the addition of the selenite. In BSO-treated cells, BSO was added to the culture (at a final concentration of 1 mM) 24 hr prior to the addition of the selenite.

RESULTS AND DISCUSSION

The hypothesis that endogenous cellular SH compounds play a role in the inhibition of RNA synthesis by selenite predicts that a decrease in the level of cellular SH compounds should result in a decrease in the inhibition of RNA synthesis by selenite. To examine this, we treated the cells with diethylmaleate (DEM), an agent that has been shown to deplete cells of SH compounds [16], and compared the effect of selenite on RNA synthesis in control and DEM-

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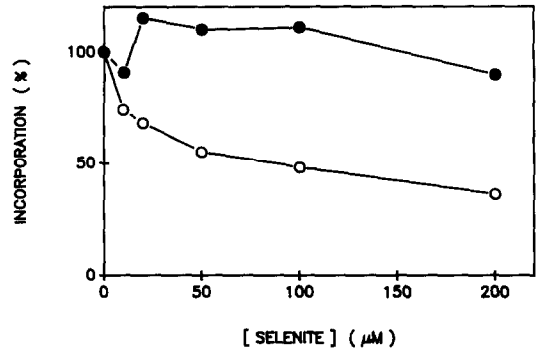

Fig. 1. Effect of selenite on RNA synthesis in SH-depleted cells. Control (○) or DEM-treated (●) cells were exposed to the indicated concentration of selenite for 1 hr. [^{3}H]Uridine (sp. act. 50 Ci/mmol) was then added to the cultures, and the incorporation of radioactivity into RNA was measured (see Materials and Methods). The results are presented as the percent of the incorporation in the absence of selenite; 100% incorporation values: (○) 3.0×10^4 cpm, (●) 1.9×10^4 cpm.

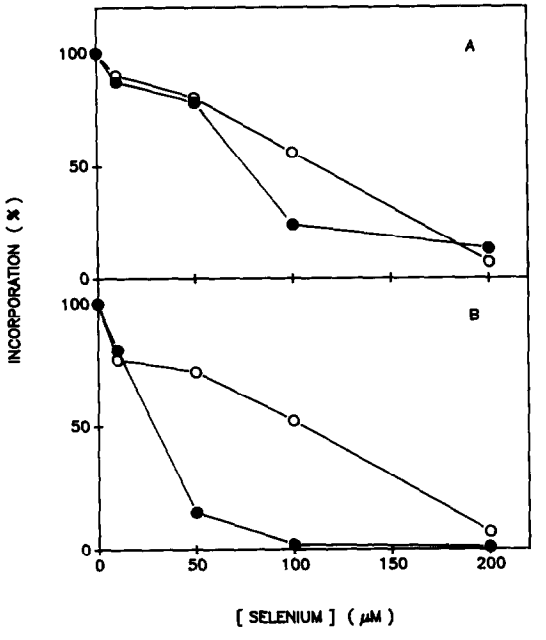

Fig. 2. Inhibition of RNA synthesis in SH-depleted cells by the product of the reaction of selenite and SH compounds. Selenite was reacted with either glutathione (A) or cysteamine (B) (at a 4:1 molar ratio of SH compound to selenite) and the reaction product was added to control (○) or DEM-treated (●) cells at the indicated final concentration of selenium. The results are presented as the percent of the incorporation in the absence of selenium. 100% incorporation values: (A): (○) 10.9×10^4 cpm, (●) 5.3×10^4 cpm; (B): (○) 9.8×10^4 cpm, (●) 4.7×10^4 cpm.

treated cells. The results (Fig. 1) show that the inhibitory effect of selenite on RNA synthesis was reduced markedly by treatment of the cells with DEM.

A reasonable explanation for this result is that, in order for selenite to inhibit RNA synthesis, it must

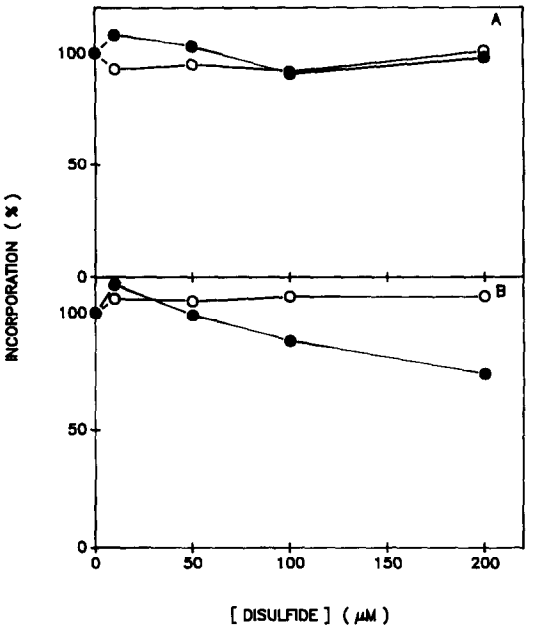

Fig. 3. Effect of disulfides on RNA synthesis. Control (○) or DEM-treated (●) cells were exposed to the indicated concentration of glutathione disulfide (A) or cysteamine disulfide (B) for 1 hr. [^{3}H]Uridine was then added to the cultures, and the incorporation of radioactivity into RNA was measured (see Materials and Methods). The results are presented as the percent of the incorporation in the absence of disulfide; 100% incorporation values: (A): (○) 2.1×10^4 cpm, (●) 0.8×10^4 cpm; (B): (○) 1.3×10^4 cpm, (●) 0.6×10^4 cpm.

first react with intracellular SH compounds to form selenotrisulfides [9]:

$$4\,RSH + H_2SeO_3 \rightarrow RSSeSR + RSSR + 3\,H_2O. \quad (1)$$

In DEM-treated cells, because of the lower concentration of SH compounds, this reaction proceeds to only a limited extent, and very little selenotrisulfide is formed. If this explanation is correct, it should be possible to overcome this deficiency by exposing the cells to selenotrisulfide rather than to selenite. To examine this, selenite was allowed to react with either glutathione or cysteamine, and the effect of the product of the reaction on RNA synthesis was determined. The results (Fig. 2) show that RNA synthesis in the DEM-treated cells was inhibited to either the same extent or to a greater extent than in control cells. To be certain that the inhibitory effect was due to the selenotrisulfide and not the disulfide product of reaction (1), we examined the effect of the disulfides of glutathione and cysteamine on RNA synthesis. The results (Fig. 3) show that neither of the disulfides had a comparable inhibitory effect on RNA synthesis in either control or DEM-treated cells. It is thus clear that the decreased inhibitory effect of selenite in DEM-treated cells (Fig. 1) is due to the lower level of SH compounds in these cells.

We also examined the effect of DEM treatment on the inhibition of RNA synthesis after a longer

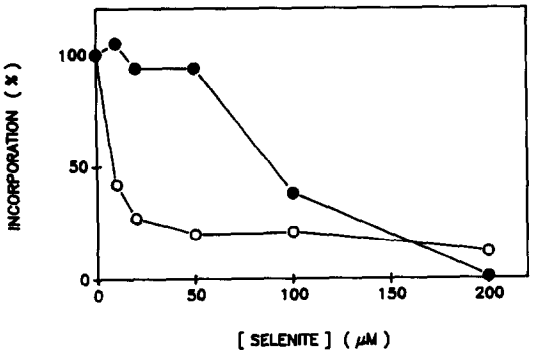

Fig. 4. Effect of a 4-hr exposure to selenite on RNA synthesis. The experiment was identical to that in Fig. 1, except that the cells were exposed to selenite for 4 hr prior to the addition of the [^{3}H]uridine. Key: control (○), and DEM-treated (●) cells; 100% incorporation values: (○) 2.1×10^4 cpm, (●) 0.3×10^4 cpm.

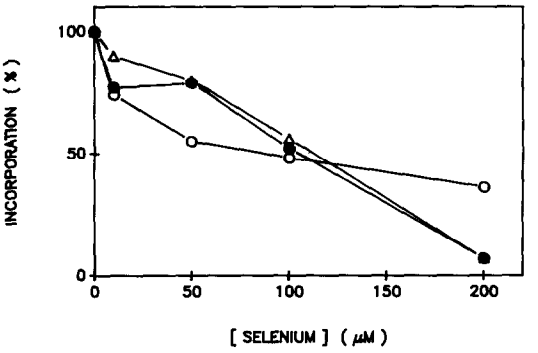

Fig. 5. Inhibition of RNA synthesis by selenite and the product of the reaction of selenite and SH compounds. The results of Figs 1 and 2 with control cells (open circles) are replotted to compare the effect of selenite (○), selenodiglutathione (Δ) and selenodicysteamine (●) on RNA synthesis in cells with normal levels of SH compounds. 100% incorporation values: (○) 3.0×10^4 cpm, (●) 9.8×10^4 cpm, (Δ) 10.9×10^4 cpm.

exposure of the cells to selenite. In control cells (Fig. 4, open circles) a 4-hr exposure to selenite resulted in greater inhibition of RNA synthesis than a 1-hr exposure (cf. Fig. 1, open circles) (50% inhibition at less than 10 μM vs approximately 100 μM). RNA synthesis in DEM-treated cells was still much less sensitive to inhibition by selenite; there was some inhibition, but only at higher concentrations of selenite. This result indicates that, under conditions of limiting cellular SH compounds, longer exposure to high concentrations of selenite can result in inhibition of RNA synthesis. This is consistent with a bimolecular reaction between the two compounds being the limiting factor in the inhibition.

If the involvement of SH compounds is, in fact, via a reaction with selenite such as shown in Eqn 1, then it might be expected that, even in cells with normal levels of SH compounds, selenotrisulfides will have a greater inhibitory effect on RNA synthesis that selenite itself. To facilitate such a comparison, the data of Figs 1 and 2 with control cells were replotted in Fig. 5. The comparison suggests that, in cells with normal levels of SH compounds, the inhibitory effect of selenotrisulfides on RNA synthesis was similar to that of selenite. This may indicate that SH compounds are normally present in cells in excess, such that the limiting factor in reaction (1) is selenite, not the SH compound; only after depletion of the cells to a level where the SH compounds were limiting was there an enhancement of inhibition by prior reaction of selenite with SH compounds.

DEM is a non-specific reagent in that it reduces the cellular level of all non-protein SH compounds [17]. Since glutathione is by far the most abundant non-protein SH compound in eucaryotic cells [18], it was of interest to examine specifically its possible role in the inhibition of RNA synthesis by selenite. To do this we treated the cells with buthionine sulfoximine (BSO), an agent which depletes the cells specifically of glutathione by inhibiting its biosynthesis [19]. The experiment in Fig. 6 shows a comparison of the effect of selenite on RNA synthesis in control and BSO-treated cells (open and closed circles, respectively). In contrast to the effect of DEM, treatment of cells with BSO did not decrease the inhibition of RNA synthesis by selenite and may have even enhanced it somewhat. Furthermore, treatment of cells with DEM in addition to BSO (Fig. 6, open triangles) had the same effect on the inhibition by selenite as it did on cells that were not treated with BSO (c.f. Fig. 1, closed circles). Thus, non-specific depletion of cells of SH compounds virtually eliminates the inhibition of RNA synthesis by selenite, but specific depletion of the cells of glutathione does not decrease the inhibition.

The simplest explanation of our results is that a cellular SH compound plays a role in the inhibition of RNA synthesis by selenite, probably by reacting with selenite to form the selenotrisulfide. However, glutathione, despite the fact that it is the most abundant cellular non-protein SH compound, does not appear to be involved in this effect of selenite. It is important to realize that the inhibition of RNA synthesis by exogenous selenodiglutathione was the same in control and DEM-treated cells (Fig. 2A). This demonstrates that glutathione can serve as the necessary SH compound in the inhibition of RNA synthesis by selenite. Our results with BSO (Fig. 6) suggest that it apparently does not do so in the cell. This may indicate that, for some reason, selenite is unable to react with glutathione intracellularly. Other studies which we have carried out [14] have indicated that the same conclusion holds true for the inhibition of DNA synthesis by selenite. This conclusion leaves open the question of which cellular SH compound or compounds are actually involved in the inhibition of nucleic acid synthesis by selenite; this is currently under investigation.

Selenium compounds are known to affect many cellular processes and functions [20], and a number of these effects have been shown to be influenced by exogenous SH compounds [1–8]. Our demonstration that cellular SH compounds are involved in the inhibition of nucleic acid synthesis by selenite leads us to hypothesize that interaction with cellular SH com-

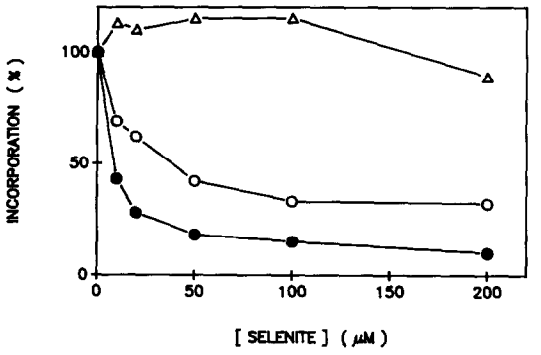

Fig. 6. Effect of selenite on RNA synthesis in glutathione-depleted cells. The experiment was carried out as in Fig. 1 with control cells (○), cells treated with BSO (●) or cells treated with DEM and BSO (△). The results are presented as the percent of the incorporation in the absence of selenite; 100% incorporation values: (○) 3.8×10^4 cpm, (●) 2.5×10^4 cpm, (△) 1.0×10^4 cpm.

pounds may be a general mechanism for the effects of selenite on cells. Of particular interest in this regard is the inhibitory effect of selenite on cell proliferation [20], which has been suggested as a possible mechanism of its anti-carcinogenicity [21, 22]. Our results do not address this question directly, since a direct relationship between the inhibition by selenite of nucleic acid synthesis and cell proliferation has not been established. Accordingly, we are currently investigating the involvement of SH compounds in the inhibitory effect of selenite on cell proliferation.

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