Effects of Selenium Compounds on Phospholipid/Ca2+-dependent Protein Kinase (Protein Kinase C) System from Human Leukemic Cells

Huai-De Su, Mamoru Shoji, Gonzalo J. Mazzei, William R. Vogler, and J. F. Kuo

Departments of Pharmacology [H-D. S., G. J. M., J. F. K.] and Medicine (Hematology and Oncology) [M. S., W. R. V.], Emory University School of Medicine, Atlanta, Georgia 30322

ABSTRACT

Selenium compounds (selenium dioxide, selenious acid, and selenic acid) were found to inhibit phospholipid/Ca2+-dependent protein kinase (protein kinase C) with the phorbol ester-stimulated phosphorylation of endogenous substrate proteins from HL60 cells. Kinetic analysis indicated that selenium dioxide (SeO2) inhibited the enzyme noncompetitively with respect to phosphatidylserine (apparent Kd 60 µM) and Ca2+ (apparent Kd 68 µM). The inhibitory effect of SeO2 on protein kinase C was additive to that of another inhibitor of the enzyme (alkyl-lysophospholipid) when present together. SeO2 was also equally inhibitory to myosin light chain kinase, a calmodulin/Ca2+-dependent class of protein kinase. It, however, affected only very slightly cyclic adenosine 3′:5′-monophosphate-dependent protein kinase. It is suggested that inhibition of Ca2+-dependent reactions might be related to the anticarcinogenic property of selenium.

INTRODUCTION

The biological importance of the trace element selenium has been recognized since 1957 (1, 2). The more recent evidence indicates that selenium is an essential nutrient, as indicated by its apparently specific role in preventing the degeneration of exocrine pancreas in chicks (3) and by its presence in glutathione peroxidase as an enzyme constituent and activator (4). The hepatic activity of glutathione peroxidase was markedly lower in the selenium deficient group of mice (maintained on diet containing less than 0.01 ppm selenium) compared to the selenium-adequate group (maintained on diet supplemented with 0.5 ppm selenium) (5). The decreased enzyme activity, however, was recovered upon dietary supplement of selenium (6). A multifocal myocarditis (Keshan disease) and a disorder of cartilage development (Kashin-Beck disease) in children have been reported to occur in certain regions of China, where the selenium intake is low as evidenced by low selenium blood levels of about 0.02 µg/ml compared to the normal levels of about 0.1-0.4 µg/ml (7, 8). It is not clear, however, whether the selenium-deficient human disorders are primarily related to the decreased glutathione peroxidase activity or other unknown metabolic functions of the element. High selenium (e.g., blood selenium level of about 3 µg/ml), on the other hand, has been shown to cause endemic selenosis in humans (7) and various diseases in animals (9).

Another interesting property of selenium is its anticarcinogenic effect. Supranutritional levels of selenium have been shown to inhibit the initiation and promotion stage of chemical carcinogenesis (10, 11), the development of nonchemically induced tumors (12-14), and the growth of hepatoma cells (15). Although the molecular mechanisms underlying the antiproliferative effect of selenium are not entirely clear, it is likely that its reported ability to increase the ratio of oxidized to reduced glutathione (15), to inhibit protein (16, 17) and DNA (16, 18, 19) synthesis, and to potentiate immune responses (20) might be partly responsible.

Protein kinase C, originally discovered in rat brain (21) and subsequently found to occur ubiquitously in the animal kingdom (22), is a key protein phosphorylation system shown to play a pivotal role in membrane signal transduction, cell regulation, and tumor promotion (23). Furthermore, the enzyme has also been shown to be a site of actions of many agents with diverse molecular sizes and structures (for a recent review, see Ref. 24). These agents include certain anticancer compounds, such as Adriamycin (25), ALP (26), lipidial amine CP-46,665-1 (27), and tamoxifen (28). In the present studies we report that selenium, the trace element nutrient with an anticarcinogenic property as mentioned earlier (10-15), also inhibited the protein kinase C system at concentrations shown by others to inhibit the growth of the cultured hepatoma cells (15).

MATERIALS AND METHODS

Materials. SeO2 and selenious acid (H₂SeO₄) were purchased from J. T. Baker Chemical Co. (Phillipsburg, NJ); selenic acid was from Pfaltz and Bauer, Inc. (Stanford, CT); selenium dioxide, selenocystamine, histone H1 (lysine rich, type III-S), mixed histone (type II), phosphatidylserine (brain), and tamoxifen were from Sigma Chemical Co. (St. Louis, MO); calmodulin was from Sciojen Corp. (Detroit, MI); TPA was from L C Services Corp. (Woburn, MA); media, sera, and antibiotics for the growth of HL60 cells were from Grand Island Biological Co. (Grand Island, NY). Other chemicals used are reagent grades. ALP (1-octadecyl-2-methyl-sn-glycerol-3-phosphocholine) was kindly supplied in solid form by Dr. Paul Munder, Max Plank Institute for Immunobiology (Freiburg, Federal Republic of Germany). The HL60 human promyelocytic leukemia cell line was purchased from the American Type Culture Collection (Rockville, MD).

Methods. Acute myelocytic leukemia cells were obtained from leukemia patients undergoing therapeutic leukapheresis at Emory University Hospital. The cells were frozen at −70°C for 2-6 months. Protein kinase C was partially purified from the total, solubilized fraction of acute myelocytic leukemia cells as described previously (26). The enzyme was also partially purified from pig brain extracts through the DEAE-cellulose step (29) or purified to homogeneity through the phosphatidylserine affinity step (30). For comparison, the enzyme from pig heart extracts was also partially purified through the Sephacryl S-200 step (29) or affinity purified to apparent homogeneity (30). MLCK from pig heart extracts was purified to apparent homogeneity according to the procedures of Walsh et al. (31). PKA was partially purified from bovine lung extracts through the DEAE-cellulose step (32).

Protein kinase C was assayed as described elsewhere (25-30). Briefly, the standard reaction mixtures contained, in 0.2 ml, 5 µmol of 1,4-piperazineethanesulfonic acid (pH 6.5), 2 µmol of MgCl₂, 40 µg of histone H1, 5 µg of phosphatidylserine, 0.04 µmol of ethylene glycol bis(β-aminoethyl ether)-N,N′,N″,N‴-tetraacetic acid, 1 nmol of [γ-³²P]-ATP (containing about 1 × 10⁶ cpm), with or without 0.1 µmol of CaCl₂, and appropriate amounts of the enzyme. The reaction was...
carried out at 30°C for 5 min. APK, using mixed histone as substrate (32), and MLCK, using cardiac myosin light chain as substrate (25, 31), were assayed as described in the references cited. The total, solubilized fraction of HL60 cells was prepared as described previously (26). For the phosphorylation of endogenous substrate proteins, 100–200 μg of cell protein were incubated under the standard assay conditions for protein kinase C mentioned above, but in the presence of various additions as indicated in Fig. 5. The subsequent sodium dodecyl sulfate-polyacrylamide gel electrophoresis and autoradiography of the phosphorylated proteins were performed as described (25, 28). In all experiments reported herein, the phosphorylation reaction catalyzed by protein kinases was linear as a function of incubation time and enzyme amount. [γ-32P]ATP was prepared according to the method of Post and Sen (33).

RESULTS

Inorganic selenium compounds, such as SeO₂, selenious acid, and selenic acid, were found to inhibit leukemic cell protein kinase C, with the concentrations causing a 50% inhibition ranging from 45 to 60 μM (Fig. 1). Selenocystamine, an organic selenium compound, on the other hand, was not inhibitory at a concentration as high as 500 μM. It appeared that the active selenium compounds inhibited the Ca²⁺-dependent enzyme activity (in the presence of phosphatidylserine) more markedly than they did the basal enzyme activity.

The inhibitory effect of SeO₂ was not unique to the leukemic cell protein kinase C, because it similarly inhibited the enzyme preparations of different purities from the pig brain and heart (Table 1). SeO₂ was also equally inhibitory to MLCK, a calmodulin/Ca²⁺-dependent class of protein kinase. In this respect SeO₂, like many other agents such as trifluoperazine (34) and tamoxifen (28), can be regarded as a general inhibitor of Ca²⁺-dependent enzymes, because it inhibited the two classes of enzymes utilizing either phosphatidylserine or calmodulin as a cofactor. SeO₂, however, affected APK only very slightly.

The kinetic analysis indicated that SeO₂ inhibited protein kinase C noncompetitively with respect to phosphatidylserine, with an average apparent Kᵢ of 60 μM (Fig. 2). It also inhibited the enzyme noncompetitively with respect to CaCl₂, with an average apparent Kᵢ of 68 μM (Fig. 3). We reported previously that many inhibitors of protein kinase C examined to date inhibited the enzyme competitively with respect to phosphatidylserine but noncompetitively with respect to Ca²⁺. These agents, for example, include tamoxifen (28), ALP (26), poly-

Table 1  Comparative effects of SeO₂ on various protein kinases

<table>
<thead>
<tr>
<th>Kind, source, and amount of protein kinase</th>
<th>Enzyme activity (pmol P/ min) at following concentrations of SeO₂</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Protein kinase C (pig brain, 18 μg)</td>
<td>18.4</td>
</tr>
<tr>
<td>Protein kinase C (pig brain, 0.01 μg)</td>
<td>4.7</td>
</tr>
<tr>
<td>Protein kinase C (pig heart, 12 μg)</td>
<td>2.9</td>
</tr>
<tr>
<td>Protein kinase C (pig heart, 0.01 μg)</td>
<td>4.2</td>
</tr>
<tr>
<td>Protein kinase C (human acute myelocytic leukemia cells, 21 μg)</td>
<td>17.3</td>
</tr>
<tr>
<td>MLCK (pig heart, 0.01 μg)</td>
<td>4.5</td>
</tr>
<tr>
<td>APK (bovine lung, 28 μg)</td>
<td>21.2</td>
</tr>
</tbody>
</table>

* Partially purified through the DEAE-cellulose step (29).
* Purified to homogeneity through the phosphatidylserine affinity step (30).
* Purified to homogeneity as described by Walsh et al. (31).
* Partially purified through the DEAE-cellulose step (32).

Fig. 1. Comparative effects of selenium compounds on protein kinase C from human acute myelocytic leukemia cells. The enzyme (21 μg) was assayed under the standard conditions in the presence of varying concentrations of the compounds, as indicated. The values indicated are the averages of duplicate assays, with assay errors being less than 5%. The findings have been confirmed in two other separate experiments. Other experimental conditions were described under "Materials and Methods."
myxin B (34), and trifluoperazine (35).

Next, we investigated the inhibitory effect of SeO2 on protein kinase C when it was present in combination with other inhibitors of the enzyme. An apparent additive inhibition was noted when SeO2 was present together with ALP but not with tamoxifen (Fig. 4). The simple additive effect of SeO2 and ALP observed here was similar to that reported previously for the combination of palmitoylcarnitine and Adriamycin (35), but it was distinct from a marked synergistic inhibitory effect seen when palmitoylcarnitine was used in combination with trifluoperazine (35).

In addition to the inhibition of protein kinase C phosphorylation of histone H1 shown above (Figs. 1–4; Table 1), SeO2 also affected phosphorylation of various endogenous proteins for the enzyme from HL60 cells. SeO2 inhibited, in a dose-dependent manner, the phosphatidylserine/Ca2+/TPA-stimulated phosphorylation of many proteins, notably those species corresponding to M, 100,000, 95,000, 67,000, 58,000, 46,000, 41,000, 23,000, and 18,000 (Fig. 5). Phosphorylation of other proteins (notably the species M, 36,000), the phosphorylation of which was independent of phosphatidylserine/Ca2+/TPA, in comparison, was only slightly affected by SeO2. The potency of SeO2 inhibition of endogenous phosphorylation was lower compared to that seen for histone phosphorylation (Fig. 1; Table 1). The likely reasons are the presence of SeO2-binding substances in the solubilized fraction of HL60 cells and antagonism of TPA on SeO2 inhibition.

**DISCUSSION**

Tumor promoters TPA (36) and mezerein (37), like the putative second messenger diacylglycerol (38), have been shown to directly stimulate protein kinase C. Because of the potential role of protein kinase C in cell growth, the sustained activation of the enzyme afforded by the nonmetabolizable tumor promoters may represent a plausible basis for their tumorigenic effects. We have reported recently that certain antineoplastic agents, such as Adriamycin (25), ALP (26), the lipoidal amine CP-46,665-1 (27), and tamoxifen (28), and the selenium in the present studies effectively inhibited protein kinase C. It is worth noting that a comparable potency was observed for ALP to inhibit the enzyme (26) and the growth of human leukemic cells (39), for tamoxifen to inhibit the enzyme (28) and the growth of human ovarian carcinomas (40), and for selenium compounds to inhibit the enzyme (present studies) and the growth of hepatoma cells (15). The present studies (Fig. 5) showed also that SeO2 countered the stimulatory effect of TPA on phosphorylation of leukemic cell proteins. The exact causal relationship between protein kinase C inhibition and the antiproliferative action of the agents, however, remains to be established. Inhibition of protein kinase C by selenium shown in the present studies demonstrated that the enzyme was a hitherto unrecognized site of action of the element. The selenium compounds, like other inhibitors, could be useful in the identification of specific substrate proteins for the enzyme, some of which could serve as potential markers for certain cancer cells. Furthermore, elucidation of the basic mechanisms of carcinogenesis could be potentially aided by investigating the biological activities and the regulatory properties of these substrate proteins unique to cancer cells.

**REFERENCES**

SELENIUM INHIBITION OF PROTEIN KINASE C


Effects of Selenium Compounds on Phospholipid/Ca^{2+}-dependent Protein Kinase (Protein Kinase C) System from Human Leukemic Cells

Huai-De Su, Mamoru Shoji, Gonzalo J. Mazzei, et al.


Updated version

Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/46/7/3684

E-mail alerts

Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions

To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions

To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.