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A Cell Surface Glycoprotein Virus Inhibitor that is Not Interferon

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SUMMARY

The possible relationship between a newly isolated glycoprotein virus inhibitor and interferon was assessed. Comparisons of the cell surface glycopeptide, obtained from mouse cerebral cortex, and interferon included antiviral activity, radioimmune assays, and the ability of antibodies raised against the brain cell surface glycoprotein (BCSG) and against mouse L cell interferon to precipitate the biological activity. BCSG was able to inhibit virus replication but only in a transient fashion. Although anti-BCSG precipitated a major portion of the radiolabelled inhibitor in a double antibody assay, anti-mouse interferon did not. Over 90% of the inhibitory activity was removed with anti-BCSG and Staphylococcus protein A while anti-mouse interferon removed little, or none, of the activity under similar reaction conditions. Other properties of the BCSG that distinguish it from interferon are presented.

INTRODUCTION

We have isolated and partially purified a naturally occurring cell surface glycopeptide from mouse cerebral cortex that inhibits cellular protein synthesis and replication (1,2). The inhibitor is active at nanogram levels, nontoxic, and fully reversible (2,3). In addition to its inhibitory activity to cell metabolism and growth, the brain cell surface glycopeptide (BCSG) has been shown to effectively inhibit vesicular stomatitis virus (VSV) protein synthesis at the level of polypeptide chain elongation while VSV-directed RNA synthesis continued at normal levels (4).

Since interferons are glycoproteins with both antiviral and cell growth inhibitory activities (5,6), the possible relationship of BCSG and mouse

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interferon was assessed. Preparations of interferon, for example, have been reported to inhibit cell multiplication (7,8), protein synthesis (9,10), thymidine incorporation (11), and numerous other cell functions, In light of the similar inhibitory activities of BCSG, it was important to determine if this cell surface inhibitor was related to mouse interferon or whether we have isolated a new class of cell and virus inhibitor.

MATERIALS AND METHODS

Isolation and Purification of BCSG. The glycopeptide inhibitor was prepared from cerebral cortex cells of 6 week-old Swiss albino outbred mice by mild Pronase treatment as described previously (2). The macromolecules released by proteolysis were concentrated and subjected to Bio-Gel P-100 filtration. The high molecular weight inhibitor (apparent mol. wt. 30,000) was used in this study (2).

Cell Cultures and Virus Stocks. Baby hamster kidney cells (BHK-21) and BHK-21 cells transformed by polyoma virus (pyBHK) were grown as described previously (3). Cells were routinely grown as monolayer cultures at 37°C in a 5% CO_2: 95% air atmosphere in humidified incubators. All medium components were purchased from Grand Island Biological Company (Grand Island, NY). The Indiana strain of VSV was kindly provided by Dr. M.E. Reichman (Univ. of Illinois, Urbana, IL). Stocks of VSV were grown in BHK-21 cells and infectious B particles were prepared by sucrose gradient purification (12).

VSV Growth Assays. Suspensions of 2×10^6 cells/ml of either BHK-21 or pyBHK were infected with VSV at a multiplicity of 10 plaque forming units/cell. After 30 min of adsorption at room temperature the cells were washed with 10 ml of Hanks' balanced salt solution (HBSS) and pelleted by centrifugation at 1,000 x g. The cells were washed twice more with HBSS and then resuspended to 1×10^5 cells/ml with DMEM containing either 40 µg BCSG or no inhibitor (controls). The test tubes were then sealed and the infected cell suspensions were incubated in a 37°C water bath for 0, 3, 5 and 10 hrs. Virus yields were determined by plaque assay on BHK-21 cell monolayers (12). In some cases BCSG was directly added to VSV-infected BHK-21 cell monolayers to test the ability of BCSG to reduce plaque formation.

Antisera. Anti-BCSG was prepared from immunized BALB/c mice. Blood was obtained from hyperimmunized mice by tail bleeding, clotted, the sera was decomplemented, and then clarified by centrifugation at 10,000 x g for 1 hr. Rabbit anti-mouse (L cell) interferon was kindly supplied by Dr. June K. Dunnick of the Antiviral Substances Program, National Institutes of Allergy and Infectious Diseases, HHS.

Radioimmune Assays. BCSG was radioiodinated to a specific activity of 6,000 cpm/ng by the method of Wood et al. (13). Radioiodination did not appreciably decrease the biological activity of the BCSG. The ^{125}I-BCSG was reacted with 2.5 to 20.0 µl of anti-BCSG or anti-mouse interferon (diluted 1:10 in pH 8.0 saline-borate buffer (133 mM NaCl, 162 mM boric acid) for 16 hr at 4°C. Anti-mouse IgG or anti-rabbit IgG (Miles Laboratories, Elkart, IN) was diluted 1:10 in saline-borate buffer and added (5 to 20 µl), after which volumes were brought to 100 µl with saline-borate buffer. The samples were further incubated at 4°C for 48 hr and the immune complexes were then

pelleted at 10,000 x g and the radioactivity in the resulting pellets determined.

Immune Precipitation of the Biological Activity of BCSG. BCSG (0.8 to 2.0 µg in a 25 µl volume) was incubated with anti-BCSG or anti-mouse interferon for 60 min at 4°C. An equal volume of insolubilized Staphylococcus protein A (PA; 20 mg/ml suspended in saline-borate buffer) was added to each tube and the mixtures were incubated for 60 min at 4°C. The PA:antibody complexes were pelleted by centrifugation at 5,000 x g for 30 min and the supernatant fluids were collected and assayed for their ability to inhibit BHK-21 cell protein synthesis. Controls included BCSG incubated with PA alone, to verify that PA itself did not remove biological activity; PA with anti-BCSG or anti-mouse interferon (without BCSG), to verify that a non gamma globulin in the sera did not influence BHK-21 cell protein synthesis; and PA reacted with anti-BCSG and anti-mouse interferon where the insoluble complexes were removed by centrifugation and then BCSG added to the supernatant fluids, to verify that no components in the antisera other than IgG could bind or inactivate BCSG.

Protein Synthesis Assays. Supernatant fluids, containing 0.1 to 4.0 µg of BCSG, obtained from PA-antibody reactions were added to test tubes and brought to a final volume of 25 µl with HKM buffer (20 mM Hepes; 120 mM KCl; 5 mM $MgCl_2$; pH 7.1). BHK-21 cells (1 x 10^6) in DMEM-Hepes were added to each tube which were then preincubated for 45 min at 37°C and protein synthesis was measured with ^{35}S-methionine for 60 min at 37°C (1).

RESULTS

The presence of 40 µg/ml of BCSG inhibited VSV replication in BHK-21 cells, although the inhibition appeared to be temporary, and by 3 hr post-infection the virus escaped the inhibitory action (Fig. 1). VSV replication was only slightly inhibited by 20 µg/ml of BCSG and not inhibited at all by 10 µg/ml (data not shown). The amount of BCSG required to inhibit VSV is in marked contrast to BHK-21 cell sensitivity to BCSG, since, at the stage of Bio-Gel 200 purification, cellular replication can be completely arrested with 10 µg/ml of the inhibitor (3). In addition to single step virus growth assays, we tested the ability of BCSG to reduce VSV plaque formation on BHK-21 monolayers. No reduction in plaque number or size was observed with as much as 150 µg/ml of the inhibitor. In other experiments we have tested the ability of BCSG to inhibit VSV replication in pyBHK cells. Unlike BHK-21 cells, the presence of BCSG at 40 µg/ml had no inhibitory effect on VSV replication in pyBHK. These observations are consistent with the primary action of BCSG being mediated at the cellular level and

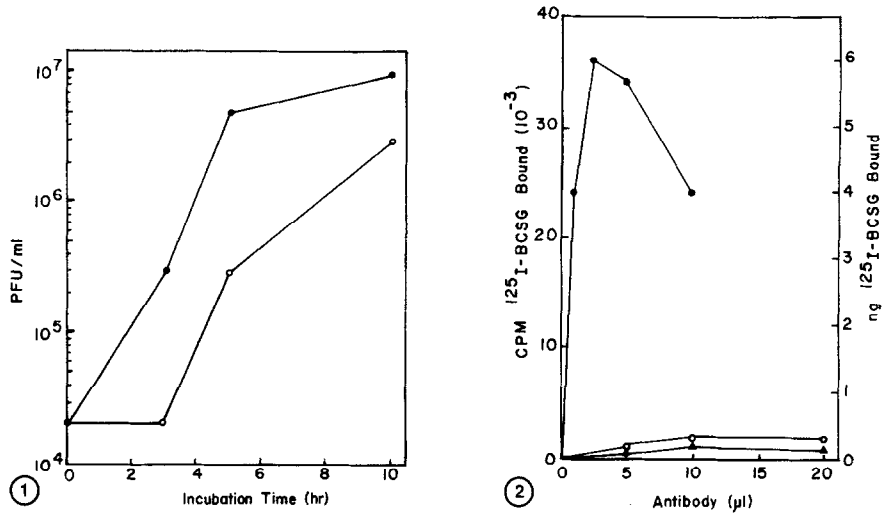

Fig. 1. Inhibition of VSV Replication by BCSG. BHK-21 cells were infected with VSV at a multiplicity of infection of 10 as described in the Materials and Methods. The infected cells were incubated in DMEM without BCSG (●) or with 40 µg/ml of BCSG (○). Virus yields were determined by plaque assay on BHK-21 cell monolayers.

Fig. 2. Precipitation of ^{125}I-Labelled BCSG with Anti-BCSG and Anti-Mouse Interferon Antibodies. ^{125}I-BCSG (15 ng) was incubated with 5 µl anti-BCSG (●) or 5 µl (○) or 20 µl (▲) of rabbit anti-mouse interferon as described in the Materials and Methods. The immune complexes were then precipitated with rabbit anti-mouse IgG or goat anti-rabbit IgG for 48 hr at 4°C. The radioactivity precipitated by this double antibody reaction was directly measured in the pellets after centrifugation and the ng of BCSG precipitated was calculated from the specific activity.

the refractory nature of pyBHK and other transformed cell lines to the inhibitory activity (1,3).

In order to assess a possible antigenic relatedness of BCSG and mouse interferon, radioiodinated BCSG was reacted with anti-BCSG or anti-mouse interferon in a double antibody assay. Although anti-BCSG removed 40% of the radiolabelled glycoprotein from solution, anti-mouse interferon removed no more than 2% (Fig. 2). This clearly indicated that the bulk of the radiolabelled material in the BCSG preparation was not reactive with the anti-mouse interferon. It was still possible, however, that the small amount of radioactively labelled BCSG, precipitated by the anti-mouse interferon antibody, was related to the biologically active inhibitor. To test this possibility BCSG was reacted with anti-BCSG or anti-mouse interferon, the immune complexes were precipitated with PA, and the inhibitor

Table 1. Immune Precipitation of the Inhibitory Activity of BCSG Preparations.

Antibody	BCSG Concentration (μg)	Inhibitory Activity Remaining (%)
None	0.8	100 ± 4
	1.2	96 ± 6
	2.0	96 ± 5
Anti-BCSG	0.8	0
	1.2	1
	2.0	0
Anti-mouse interferon	0.8	88 ± 8
	1.2	88 ± 9
	2.0	93 ± 4

Various concentrations of BCSG were incubated with either anti-BCSG or anti-mouse interferon antibody and PA as described in the Materials and Methods. Following centrifugation at 10,000 x g to remove the immune complexes, the inhibitory activity remaining in the supernatant fluids was measured with the protein synthesis assay with BHK-21 cells. The results are expressed as the % of inhibitory activity remaining ± S.D. of triplicate determinations. Anti-BCSG, anti-mouse interferon or PA alone did not influence BHK-21 cell protein synthesis.

remaining in the supernatant fluid was assayed against BHK-21 cell protein synthesis. The anti-BCSG antibody and PA removed 96% to 100% of the biological activity while the anti-mouse interferon and PA removed very little, if any of the inhibitor (Table 1). These results are consistent with the antibody-radiolabelled BCSG experiments and illustrated that the inhibitory substance in the BCSG preparations was not antigenically related to mouse interferon.

DISCUSSION

Several lines of evidence illustrate that BCSG, although able to inhibit both VSV and cell protein synthesis, is not related to mouse cell interferon. Unlike mouse interferon, BCSG has not displayed a species specificity since mouse, hamster, and rat cells are equally sensitive to the inhibitor (1,3). Interferon has been shown to inhibit cellular protein synthesis but the amount of interferon required to inhibit cells was 500 to 800-fold higher than anti-viral levels (9). In contrast to interferons, the inhibitory activity of BCSG is at least four times more effective to

cells than VSV (3,4) and even then the inhibitory action of BCSG in VSV replication is temporary (Fig. 1) while cell growth is arrested for over 24 hr (3). In addition, host cell protein synthesis inhibitory activity of mouse interferon was not manifested in heterospecific cell lines (9). Unlike BCSG, mouse interferon is capable of mediating inhibitory activity in BHK-21 cells infected with polyoma virus (14). BCSG is effective against numerous primary and cell culture adapted cell lines but transformed cells including mouse neuroblastoma, pyBHK, and C3H fibrosarcoma cells are relatively resistant, or refractory to, the inhibitor (1,3).

Radioimmune assays with radioiodinated BCSG suggested that anti-mouse interferon IgG only could precipitate a minor fraction of the radiolabelled material. In contrast, anti-BCSG antibody precipitated 40% of the radiolabelled preparation (Fig. 2). In addition to the radioimmune assays, anti-mouse interferon and PA precipitated little of the biological inhibitory activity while anti-BCSG and PA removed 96 to 100% of the inhibitor (Table 1).

These results show that the biological activity of BCSG is not consistent with those of mouse interferons and that the glycopeptide apparently is not antigenically related to the virus inhibitors classified as interferons. It is, therefore, more likely that BCSG plays a natural role in cell growth regulation than as an inhibitor of virus infection and/or replication.

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REFERENCES

1. Kinders, R., Johnson, T. and Rachmeler, M. (1979) Life Sci. 24, 43-50.

2. Kinders, R.J., Milenkovic, A.G., Nordin, P. and Johnson, T.C. (1980) Biochem. J. 190, 605-614.

3. Kinders, R.J. and Johnson, T.C. (1981) Exptl. Cell Res. (in press).

4. Kinders, R.J., Hughes, J.V. and Johnson, T.C. (1980) J. Biol. Chem. 255, 6368-6372.

5. Stewart II, W.E. (1979) in Interferon 1979, (I. Gresser, ed.), Vol. 1, pp. 29-51. Academic Press, New York.

6. Gorden, J. and Minks, M. (1981) Microbiol. Rev. 45, 244-266.

7. Paucker, K., Cantell, K. and Henle, W. (1962) Virol. 17, 324-334.

8. Stewart II, W.E., Chudzio, T., Lin, L.S. and Wiranowska-Stewart, M. (1978) Proc. Nat. Acad. Sci. USA 75, 4814-4818.

9. Johnson, T.C., Lerner, M.P. and Lancz, G.J. (1968) J. Cell Biol. 36, 617-624.

10. Falcoff, R., Scheps, R., Sanceau, J., Catinot, L. and Falcoff, E. (1975) Biochemie 57, 1109-1111.

11. Brouty-Boye, D. and Tovey, M.G. (1977) Intervirol. 9, 243-252.

12. Hughes, J.V., Johnson, T.C., Rabinowitz, S.G. and Dal Canto, M.C. (1979) J. Virol. 29, 312-321.

13. Wood, F.T., Wu, M.M. and Gerhart, J.G. (1975) Anal. Biochem. 69, 339-349.

14. Dubecco, R. and Johnson, T.C. (1970) Virology 42, 368-374.