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Echinhibin-1 – an inhibitor of Sendai virus isolated from the venom of the snake *Echis coloratus*

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Summary

The snake venom of *Echis coloratus* was found to abolish the hemagglutinating activity, hemolytic activity and in vivo infectivity of Sendai virus. The active factor (Echinhibin-1) was purified by gel filtration on Sephadex G-50, followed by chromatography on DEAE-Sepharose and CM-Sepharose. Echinhibin-1 is a protease with a molecular weight of about 25 kDa, an isoelectric point of 7 and is stained by PAS, indicating that it is a glycoprotein. It showed a strong azocollase activity that was stable up to 68°C and at pH values of 4.5–10.5. Ten μg/ml were sufficient to abolish the hemolytic effect of the virus on human erythrocytes when incubation was at 37°C for 2 h, while 20 μg/ml abolished the hemagglutinating activity. Addition of Echinhibin-1 after the adsorption of Sendai virions onto washed erythrocytes at 4°C did not inhibit the subsequently hemolytic activity at 37°C, indicating that Echinhibin-1 interferes with virus adsorption to the cells. Of various protease inhibitors, only Na_2 EDTA and *o*-phenanthroline inhibited the antiviral activity of the purified factor, indicating that it is a metalloproteinase. In vivo, mice inoculated intranasally with the virus pretreated with Echinhibin-1 developed well and gained weight, whereas untreated virus-infected mice lost weight and died within 1 week. Intravenous administrations of the purified factor up to 80 μg/mouse produced no signs of toxicity and subcutaneous injections caused no hemorrhagic activity, while the whole venom is very hemorrhagic with an LD_{50} of 250 μg/kg for mice.

Echinhibin-1; Antiviral protease; Snake venom; Sendai virus

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Introduction

Antiviral activity may be directed at any of the stages of viral replication, the first of which is the adsorption to the host cell. Numerous agents have been proposed for the treatment of viral diseases in the past three decades. Most of these compounds interfere at a stage following penetration of the virus into the cell (see, for example Furman et al., 1986; Vrang et al., 1987; Ngan et al., 1988; Oxford et al., 1989; Tabba et al., 1989; Rooke et al., 1990; Yao et al., 1992). Current antiviral agents have demonstrated benefit only in a limited number of viral infections, and all have significant potential for toxicity (Chou and Merigan, 1986).

Snake venoms are complex mixtures of toxins and enzymes that show different activities on biological systems. Proteases are widely distributed in the venoms of Crotalidae and Viperidae (Emery and Russel, 1963; Stahnke, 1966; Tu et al., 1969; Tu, 1977; Markland, 1983). Some of the proteolytic enzymes are correlated with specific biological activities, such as hemorrhagic activity (Ovadia, 1978, 1987; Bjarnason and Fox, 1988-89), bradykinin-releasing activity (Sato et al., 1965) or thrombin-like activity (Sugihara et al., 1978). In a preliminary attempt snake venoms were used against poliomyelitis (Sanders et al., 1953, 1954): subcutaneous injections of several neurotoxoids derived from the venoms of the cobras *Naja flava* and *Naja naja* interfered with the course of experimental poliomyelitis caused by intracerebral infection with the Brunhilde or Lansing virus strain in *Macacus rhesus*. Padgett and Levine (1966) used the venoms of the cobra *Naja naja* and the krait *Bungarus fasciata* as crude enzyme preparations to rupture the outer envelope of the virions in order to study the fine structure of murine Rauscher leukemia virus.

Among various snake venoms screened in vitro in this laboratory as inhibitors of Sendai virus (Borkow and Ovadia, 1992), the snake venom of the viperid *Echis coloratus* had the strongest inhibitory effect on the hemolytic activity of Sendai virus. This viperid snake is found in the Middle East. This work deals for the first time with the isolation and characterization of the active factor from the venom of the snake *Echis coloratus*. The purified factor – Echinhibin-1 – abolishes the following activities of Sendai virus: hemagglutinating, hemolytic and in vivo infectivity.

Materials and Methods

Materials

Venom. Venom was obtained at our laboratory from healthy specimens of *Echis coloratus* kept at the Serpentarium of the Canadian Center for Ecological Zoology, Tel-Aviv University. The venom was 'milked' into separate vials, pooled, lyophilized and stored at $-20^{\circ}C$ until used.

Columns. Sephadex G-50 (superfine), CM-Sepharose and DEAE-

Sepharose were purchased from Pharmacia (Uppsala, Sweden). The gels were swollen and prepared as recommended by the manufacturer.

Virus. Parainfluenza Virus type I (Sendai virus, Z strain) was injected into the allantoic sac of 11-day-old embryonated chicken eggs, harvested 48 h after the injection, and purified by established procedures (Henis et al., 1985). The virus was resuspended in 160 mM NaCl/20 mM Tricine (pH 7.4) and stored at −70°C. Virus hemagglutinating activity was measured and expressed in hemagglutinating units (HAU). One ml of virus contained about 30 000 HAU.

Blood. Fresh human blood was obtained from the blood bank at Tel-Hashomer Hospital, Israel, and stored up to one month at 4°C. Prior to use, erythrocytes were washed with PBS (pH 7.2), and diluted to 2% (v/v) with the same buffer.

Methods

Interaction of Sendai virions with human erythrocytes (Hemolysis assay). Erythrocytes from fresh human blood were washed and diluted to 2% (v/v) with PBS (pH 7.2). One ml of these diluted erythrocytes was incubated for 15 min at 4°C with 100–300 HAU aliquot of Sendai virions. Excess virus was removed by washing twice with cold PBS (pH 7.2) and the pellet was gently vortexed and resuspended in 1 ml of PBS, pH 7.2. The mixture was incubated for 30 min at 37°C in a shaking bath (90 strokes per min) and then centrifuged at 3000 RPM. Hemolysis was determined by measuring the absorbance of the supernatant at 540 nm. The antiviral activity of the whole venom or its fractions was tested by incubation with aliquots of 100–300 HAU of Sendai virions for 2 h at 37°C before an hemolysis assay was carried out. As control, 100–300 HAU of Sendai virions were incubated with PBS (pH 7.2).

Hemagglutinating assay. Serial 2-fold dilutions of virus were made in 0.5 ml PBS (pH 7.2), and equal volumes were added from suspensions of 0.5% chicken red cells. Readings were done after 1 h and the end points were determined by a standard method of interpolation. Hemagglutinating tests were also performed with viruses treated with the venom or its fractions.

Infection of animals. Three-week-old ICR mice weighing 9–10 g were inoculated intranasally under mild ether anesthesia, with 25 μl containing 37.5 HAU of the virus. Each animal was measured daily for body weight, which was shown to be a good indication of the pneumopathogenicity of Sendai virus (Tashiro and Homma, 1983).

Hemorrhagic activity. The active antiviral component was tested by the skin injection method of Kondo et al. (1960) with some modifications of Ovadia (1978). One tenth of a milliliter of saline containing 0.05–40 μg of the examined venom material was injected into the skin at the back of white mice.

20 h later the inner surface of the removed skin was observed for hemorrhagic spots.

Proteolytic activity. General proteolytic activity was tested on azocoll according to the method of Moore (1969) modified by Ovadia (1987). Briefly, aliquots containing various amounts of the whole venom, or the different fractions, were incubated separately with 10 mg of azocoll (insoluble dye - protein complex) suspended in 2 ml of 0.1 M phosphate buffer (pH 7.2) in a shaking bath (120 strokes per min) for 1–3 h at 37°C. The reaction was stopped by centrifugation and the proteolytic activity was estimated by measuring the absorbance of the clear supernatant at 520 nm.

The hydrolytic effect on the substrate *N*-benzoyl-L-arginine ethyl ester (BAEE) was examined according to the method of Schwert and Takenaka (1955). Aliquots containing 5–100 μg of the purified venom fraction were mixed with 1 ml of 0.5 mM BAEE in 0.05 M glycine buffer, pH 9.5. Proteolytic activity at 37°C was determined by measuring the change in absorbance at 254 nm.

Proteolytic activity on gelatin was examined according to Ovadia (1978). A drop containing 0.1 mg of the purified venom factor was applied onto an undeveloped black and white film and incubated for 3 h at 37°C in a moist chamber. The film was then washed thoroughly with tap water. Gelatin digestion is indicated by a transparent spot on an opaque background. Trypsin served as a positive control.

Immunodiffusion. Immunodiffusion was carried out in 1% agar gel according to the procedure of Ouchterlony (1953). Rabbit antiserum against whole *Echis coloratus* venom was placed in the central well, and the isolated factor and the whole venom were placed in the peripheral wells at a distance of 0.8 cm. After overnight incubation at room temperature, the gels were washed three times for 3 days with saline and dried. The precipitation lines were stained with Ponceu S (0.5% in 5% TCA [Trichloroacetic Acid]) for 10 min and destaining was carried out with several changes of 5% acetic acid.

Immunoelectrophoresis. Immunoelectrophoresis was performed in 1% agar gels for 2 h at 300 V in sodium-Tris barbital buffer ($I = 0.04$, pH 8.8). The antibodies were placed in the central trough for 24 h, followed by three washings with saline for 3 days, and dried. The precipitation lines were stained as above.

Isoelectric focusing. Seven markers of known isoelectric points (pIs) and the antiviral factor were run separately in 2 ml tubes of 10% polyacrylamide gels containing 1% carrier ampholine with a pH range of 3- 10. Two hundred volts were applied overnight followed by 300 volts for 2 h. The anode solution was 1% phosphoric acid and the cathode 1% NaOH. The gels were fixed with 10% TCA and the proteins were stained by 0.025% Coomassie brilliant blue as above.

Electrophoresis. Electrophoresis was carried out according to the method of Davis (1964). The purified factor and the whole venom were applied onto 7.5% polyacrylamide slab gels. The running buffer was 0.05 M Tris-glycine (pH 8.3) and a current of 20 mA was applied. The gels were fixed with 40% methanol/10% acetic acid (v/v) and then stained with the Bio-Rad Silver Stain Kit (Bio-Rad Laboratories, 2000 Alfred Nobel Drive, Hercules, CA 94547).

Determination of molecular weight. The molecular weight was determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) according to the procedure of Laemmli (1970). Seven markers of known molecular weight, the antiviral factor, Sendai virions and a mixture of the antiviral factor with the virions were run separately in 7.5% polyacrylamide gels in the presence of 0.2% SDS dissolved in 0.1 M phosphate buffer (pH 7). The gels were fixed with 10% acetic acid containing 10% isopropanol followed by staining with 0.025% Coomassie brilliant blue for proteins or with Bio-Rad Silver Stain Kit. Staining of the gels by the PAS procedure was done as follows: the fixed gels were washed with iodinized water, followed by oxidation with 0.2% sodium metaperiodate for 45 min, thoroughly washed with iodinized water and stained with Schiff's reagent for 1 h. The relative mobility of each marker was plotted against the logarithm of its known molecular weight; the molecular size of the factor was calculated from this plot (Weber and Osborn, 1969).

Estimation and concentration of proteins. Protein content was estimated by measuring the absorbance at 280 nm in a Gilford u.v. spectrophotometer, using a value of $A\ 1\%_{280} = 14$. Protein solutions were concentrated by ultrafiltration under vacuum through a dialysis membrane (Union Carbide, Chicago, USA).

Results

Antiviral activity of Echis coloratus crude venom

The antiviral effect of the snake venom on Sendai virions was examined by hemagglutinating and hemolysis assays as described in Materials and Methods. Hemagglutinating activity of 38 500 HAU was totally lost after 2 h of incubation with 2.5 μg of crude venom in 100 μl at 37°C; 0.75 μg of the snake venom were sufficient to abolish 50% of the hemagglutinating activity. The hemolytic activity of 100 HAU of the virions was completely lost after 2 h of incubation with 10 μg of the venom in 1 ml. However, 0.15 μg of crude venom were sufficient to cause complete inhibition of the 100 HAU of virions if the incubation was prolonged for 24 h (Fig. 1).

General characterization of the antiviral factor

The following experiments were carried out in order to precharacterize the antiviral factor:

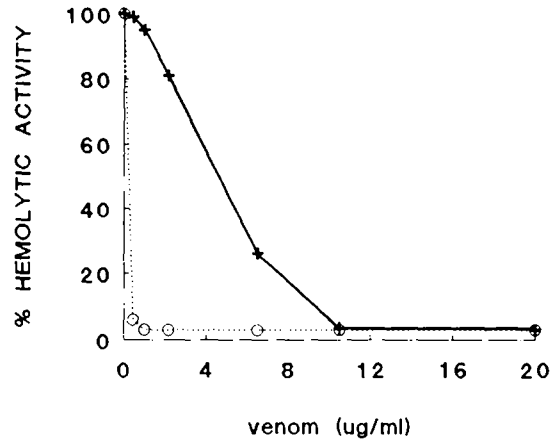

Fig. 1. Antiviral activity of the whole venom of *Echis coloratus*. Aliquots containing 100 HAU of Sendai virus in PBS were incubated with samples containing various amounts of crude venom for 2 h (–) or 24 h (○) at 37°C followed by an hemolysis assay of the mixtures as described in Methods. Controls included samples containing the virions only or the crude venom alone incubated with the erythrocytes.

(A) Digestion with trypsin. Samples containing 900 μg of the *Echis coloratus* venom in PBS were incubated with 0.25% trypsin for 1 h at 37°C, and Fetal Calf Serum (FCS) was added in order to inactivate the trypsin. Aliquots of venom incubated without trypsin, 0.25% trypsin or PBS incubated with FCS served as controls. Each of these mixtures was incubated with a sample of the virions for 2 h before the hemolysis assay was carried out. The venom abolished the viral activity completely, whereas venom preincubated with trypsin did not influence the viral activity. These results indicate that the antiviral factor is a protein which can be digested by trypsin.

(B) The thermostability of the antiviral factor. This was examined as follows: samples containing 40 μg of *Echis coloratus* venom in PBS were heated for 15 min at various temperatures and cooled before they were incubated with 300 HAU Sendai virions for 2 h at 37°C, and the mixtures were assayed for hemolysis as described. The hemolysis caused by these samples was compared with samples containing non-heated venom. The antiviral factor was found to be thermolabile; no effect was observed on antiviral activity up to 60°C, whereas its inhibitory capacity was lost for 50% after heating at 70°C, and completely after heating at 80°C.

(C) The stability of the antiviral activity at various pH values. This was examined by adding 950 μl of various buffer solutions at different pH values to 50 μl samples containing 400 μg of whole venom. The mixtures were left overnight at 4°C before they were adjusted to pH 7.2 by dialysis against PBS and were tested in hemolysis experiments as described. The antiviral activity

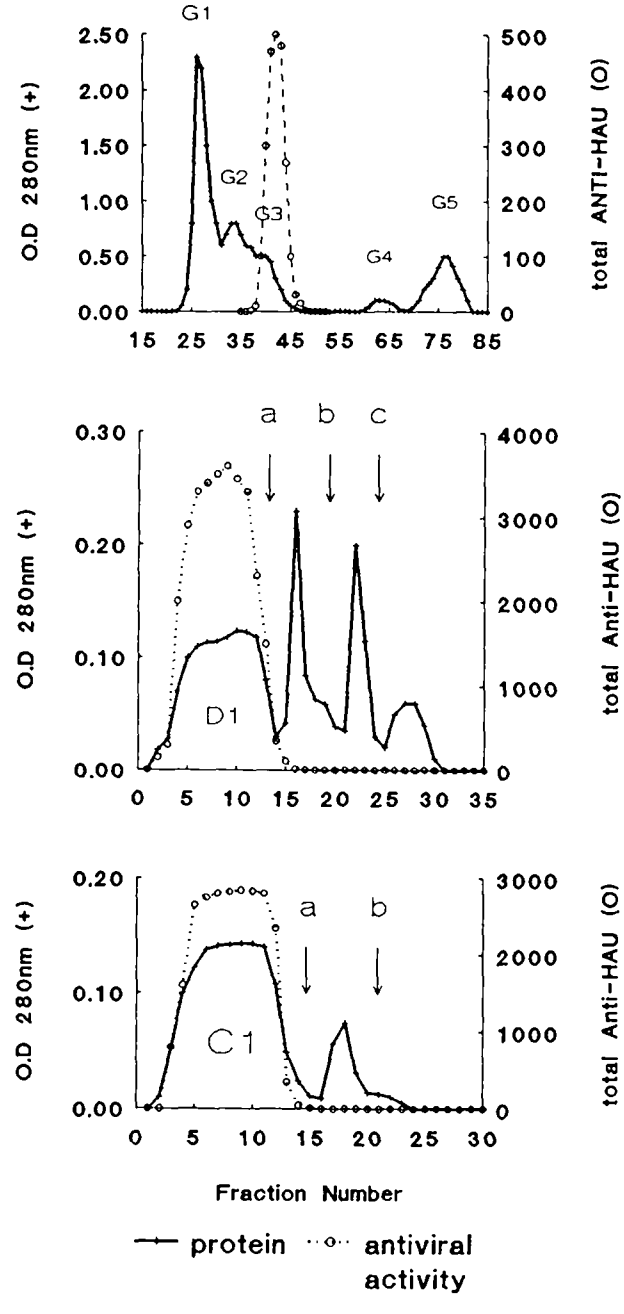

Fig. 2. Purification scheme of Echinhibin-1 from *Echis coloratus* venom using chromatographic procedures (see Materials and Methods section for complete details). (a) Sephadex G-50, (b) DEAE-Sepharose, and (c) CM-Sepharose. From each step the peak fractions containing antiviral activity (dotted lines on figures) from the previous column were pooled and then applied onto the next column. (a) 0.1 M NaCl; (b) 0.5 M NaCl; (c) 1 M NaCl.

was stable within the pH range of 4.5–10.5.

(D) The influence of phosphate buffer on the antiviral activity. This could be demonstrated by a linear curve. As the molarity of the phosphate buffer was increased in the solution, the antiviral activity of the venom decreased. No effect on antiviral activity was observed up to 0.05 M phosphate buffer; 0.175 M of phosphate buffer reduced the antiviral activity of the crude venom to 50%, while concentrations of 0.5 M or higher abolished the capacity of the venom to inhibit Sendai virus.

Purification of the antiviral factor

As the general characterization indicated that the antiviral factor is a protein, the following procedures of protein fractionation were used to purify the antiviral factor.

(i) Gel filtration on Sephadex G-50. 50 mg of lyophilized *Echis coloratus* venom was dissolved in 1 ml of phosphate buffer, 0.01 M, pH 7.2 and was centrifugated at 5000 × *g* before application on a column of Sephadex G-50, 1 × 90 cm. The elution was performed with the same buffer at a flow rate of 2 ml/cm^2/h; the effluent was collected in 2 ml fractions. The inhibitor was located in fractions 37–45 (Fig. 2a) with a 7.5-fold increase in the specific activity (Table 1).

(ii) Chromatography on DEAE-Sepharose. Fractions 37–45 of the previous step were pooled and subjected to column chromatography on DEAE-Sepharose (5 × 0.6 cm) equilibrated with 0.01 M phosphate buffer, pH 7.2. The column was washed by the same buffer before elution with a gradual increase of salt concentration, as shown in Fig. 2b. The inhibitor was eluted with the fractions of the first peak D1 that was not absorbed by the column. This peak showed a 10-fold increase in specific activity (Table 1).

(iii) Chromatography on CM-Sepharose. The main active fractions, found

TABLE 1

Purification of Echinhibin-1 from *Echis coloratus* venom

Step	Total protein (mg)	Total HAU neutralized	Yield (%)	Purification factor
Crude venom	50	500 000	100	1
Supernatant	33	495 000	99	1.5
G-50 (G3)	5.5	412 500	82	7.5
DEAE-S (D1)	3.5	350 000	70	10
CM-S (Echinhibin-1)	1.8	252 000	50	14

Total HAU neutralized was determined by incubating aliquots of Sendai virions with various concentrations of the antiviral fraction at each step of purification for 2 h at 37 C prior to the hemolysis assay.

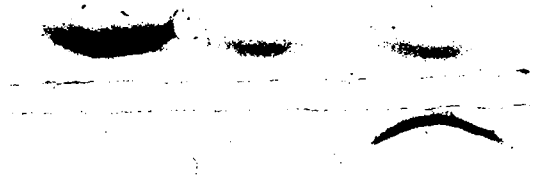

Fig. 3. Immunoelectrophoresis was performed in 1% agar gels for 3 h. The purified factor was placed in the lower well and whole venom was placed in the upper well. Antibodies against whole venom were placed in the central trough after the electrophoresis.

in the region D1 of the previous stage, were further purified by chromatography on CM-Sepharose. The fractions were pooled and applied onto a 5 × 0.6 cm column of CM-Sepharose equilibrated with 0.01 M phosphate buffer (pH 7.2). The column was eluted by a step-wise increment of various salt concentrations and the effluent was collected in 2-ml fractions (Fig. 2c). The antiviral activity was found in the first peak eluted from the column by the equilibration buffer before the salt increment. The purification factor was further raised to 14.

Homogeneity

One band appeared when the isolated venom factor was examined by immunodiffusion, immunoelectrophoresis (Fig. 3), disc electrophoresis, SDS-PAGE (Fig. 4), and isoelectric focusing with a pI of 7.

Characterization of Echinhibin-1

The purified factor was named Echinhibin-1 because it was isolated from *Echis* venom and was a potent inhibitor of the virus.

(A) The molecular weight of Echinhibin-1 was found to be 25000, as determined by SDS-PAGE (Fig. 4, lane C).

(B) Positive PAS-staining indicated that Echinhibin-1 is a glycoprotein.

(C) The following experiment indicates the importance of the incubation temperature on the activity of the antiviral factor. 300 HAU virions were incubated with various concentrations of Echinhibin-1 at 4°C, 25°C and 37°C. Fig. 5 shows that higher concentrations of the antiviral factor were needed to inhibit the hemolytic activity of the virions as the incubation temperature was lowered, which points to the enzymatic character of Echinhibin-1.

(D) The general proteolytic activity was tested on azocoll at various pH values. The optimal activity was obtained between pH values of 8.5–9.8. At pH values lower than 6 or at pH values higher than 10.5 Echinhibin-1 was not active.

(E) The effect of three types of protease inhibitors was examined by incubating them with Echinhibin-1 for 1 h at 37°C prior to their incubation with the virions. As control, Echinhibin-1 or the inhibitors alone were incubated with the virions, and an hemolysis assay was carried out as described. The antiviral

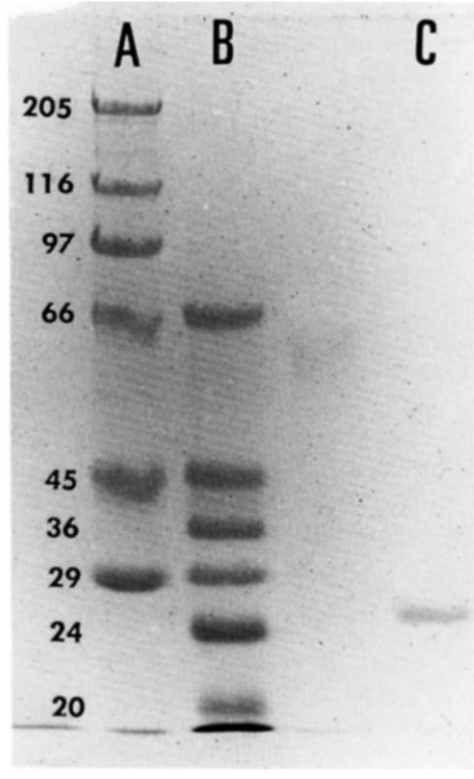

Fig. 4. Determination of purity and molecular weight of Echinhibin-1 by SDS electrophoresis gel electrophoresis performed according to the method of Laemmli (1970). Lane A: Sigma High Molecular Weight Standard mixture. Lane B: Sigma Dalton Mark VII-L molecular weight markers. Lane C: 30 μg of Echinhibin-1; Silver staining was performed after fixation.

activity was inhibited by 0.4 mM EDTA and 1 mM *o*-phenanthroline, whereas concentrations 10-fold higher than usually used for the serine protease inhibitors, a protinin (0.55 mM) and phenylmethylsulfonyl fluoride [PMSF] (1 mM) or of the acid protease inhibitor pepstatin (0.08 mM), did not affect the antiviral activity, thus indicating that the antiviral factor must be a metalloproteinase.

(F) Hemorrhagic activity: no red hemorrhagic spot were observed on the inner surface of the removed skin of white mice 20 h after the injection of as much as 40 μg of the antiviral factor, whereas 2 μg of the whole venom caused marked hemorrhagic spot.

(G) Lethal activity: 20 g ICR mice injected through the caudal vein with 0.5 ml PBS containing 80 μg of Echinhibin-1 produced no signs of toxicity for 48 h. For comparison, the LD_{50} of the crude venom was 0.25 μg/g mice.

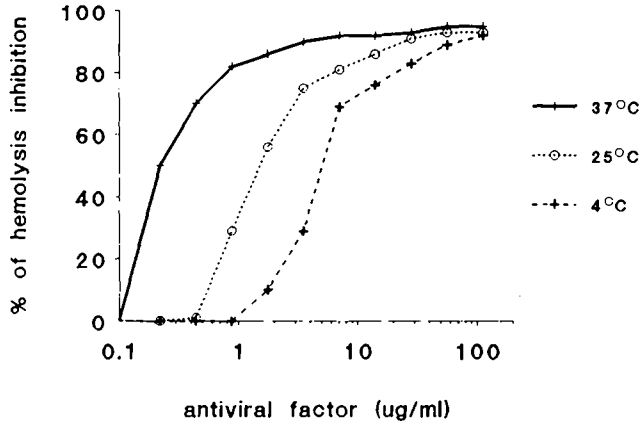

Fig. 5. Effect of incubation temperature on antiviral activity. Ten μg of the antiviral factor were incubated overnight at 4 C, 25 C and 37 C with 300 HAU virions in 100 μl of 0.01 M phosphate buffer (pH 7). An hemolysis assay was then carried out as described in the Methods section.

Neutralization of various activities of Sendai virus by Echinhibin-1

(1) Hemagglutination. The neutralization of hemagglutinating activity of the virus was examined by using a Sendai virion sample containing 38 500 HAU/ml. Echinhibin-1 reduced by 50% the hemagglutinating activity of the virus at a concentration of 5 μg/ml; at 20 μg/ml the hemagglutinating activity was completely abolished.

(2) Hemolysis. The ability to neutralize hemolysis caused by Sendai virions was examined by two series of experiments:

(a) The virus and various concentrations of the antiviral factor were incubated for 2 h or overnight at 37 C (final virus concentration 300 HAU/0.1 ml) followed by an hemolysis assay as described in Methods. One μg aliquot of the purified factor was needed to abolish the hemolysis caused by the 300 HAU virion sample if the preincubation between the purified factor and the virions lasted 2 h. However, only 0.1 μg of Echinhibin-1 was sufficient to abolish the virions activity if the preincubation between these components was prolonged overnight, indicating an enzymatic activity of the factor. To elucidate whether Echinhibin-1 inhibits the first stage of attachment of the virions onto the erythrocytes or whether it might also interfere with the second stage (fusion) that causes hemolysis of the erythrocytes, the following experiment was carried out.

(b) 300 HAU Sendai virions were incubated with 1 ml of diluted erythrocytes (2% v/v) for 1 h at 4 C. Excess virions were removed by two washes and 50 μl aliquots containing various concentrations of the antiviral factor were then added to the mixtures; the incubation was prolonged for another 2 h at 4 C or

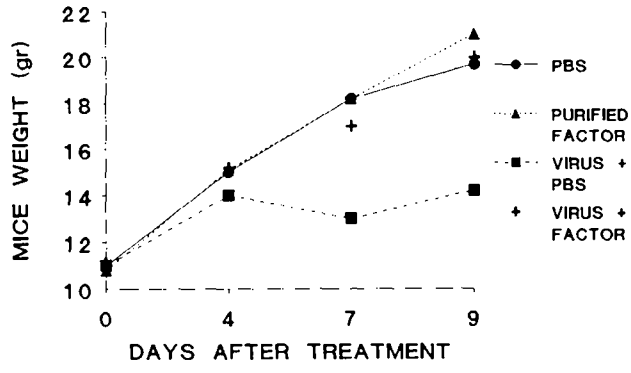

Fig. 6. Effect of pretreatment of Sendai virions with Echinhibin-1 on subsequent virus infection in mice. The body weight of mice was examined daily after intranasal inoculation with 25 μl containing 37.5 HAU of Sendai virions in PBS or of Sendai virions incubated with Echinhibin-1. PBS alone and the antiviral factor alone were injected separately as the controls.

at 37°C. The subsequent hemolysis assay revealed that no inhibition could be detected even at 10-fold higher concentrations of Echinhibin-1 than needed to abolish hemolysis in the previous experiment. Thus, Echinhibin-1 does not prevent hemolysis after attachment of the virions onto the erythrocytes has taken place. It must thus act during the first stage by inhibiting attachment of the virions onto the cells.

(3) Infection of animals. Samples (200 μl) containing 300 HAU of Sendai virions and 10 μg of Echinhibin-1 were incubated for 2 h at 37°C; 25 μl aliquots containing 37.5 HAU were then inoculated intranasally into mice. PBS alone, or virions alone or the antiviral factor alone, were inoculated intranasally to serve as controls, and the body weight of each animal was measured daily thereafter. The weight of the animals inoculated with PBS alone, or with the purified factor alone, increased normally, whereas those that had been inoculated with the virions suspended in PBS began to lose weight after the fourth day, and 5 out of 12 mice died within 1 week. Animals inoculated with the virus preincubated with Echinhibin-1 gained normal weight (Fig. 6) and none died. This result shows that the virus treated with Echinhibin-1 in vitro had been neutralized and that Echinhibin-1 is not toxic to the respiratory tract of the mouse.

Discussion

Snake venoms are composed of many components active on various biological systems (see Introduction). It has been recently reported that wounds caused by snake venoms usually show very low bacterial infection (Talan et al.,

1991). In addition, this laboratory has already shown the potential activity of various snake venoms against tumor cells and Sendai virus (Chaim-Matyas and Ovadia, 1987; Chaim-Matyas et al., 1991, 1993; Borkow and Ovadia, 1992). It is therefore important to purify the antiviral agents from the active snake venoms in order to elucidate the mechanism of their activity. The snake venom of *Echis coloratus* showed a strong inhibitory effect on Sendai virus (Borkow and Ovadia, 1992). This paper reports the first attempt to isolate and characterize the inhibitory factor from a snake venom, and demonstrates its ability to abolish the hemolytic activity, hemagglutinating activity and infectivity of the Sendai virus. The purified factor was named Echinhibin-1 because of its origin and its inhibitory effect on various activities of Sendai virus. It is a glycoprotease with a molecular weight of 25 kDa and isoelectric point of 7. Strong proteolytic activities could also be demonstrated in other fractions of the *Echis coloratus* venom, but they showed no inhibition of the Sendai virus, which indicates the specificity of Echinhibin-1. Moreover, other snake venoms which also contain high quantities of various enzymes, do not affect the hemolytic activity of the virus (Borkow and Ovadia, 1992).

It is well known that the first stage of the infectivity of parainfluenza viruses is virion attachment to the cell membranes, which is intermediated by the HN (hemagglutininneuraminidase) glycoprotein found on the virion envelope. After attachment of the virion to a host cell, the next step in infection is the delivery of the nucleocapsid into the cytoplasm. This step is mediated by a second surface glycoprotein - the fusion protein (F). This protein stems from an inactive precursor (Fo), which gains the fusion function by a cleavage process that is mediated by cellular trypsin-like enzymes (Homma and Ohuchi, 1973; Kingsbury, 1990; Gotoh et al., 1992). The inhibition of the hemolytic and the hemagglutinating activities of the Sendai virions could be achieved if Echinhibin-1 is preincubated with the free virions before they attach to the erythrocytes. After attachment, the purified factor does not influence these viral activities. It may be concluded therefore that Echinhibin-1 inhibits the first vital step of the viral infection - the adsorption of the virions to the cells, probably by specific cleavage of the HN protein, a possibility that should be further examined.

Previous studies also revealed proteases, isolated from various organisms, which specifically remove the hydrophobic anchor peptides from the viruses (Reginster, 1965; Biddle, 1968; Compans et al., 1970; Brand and Skehel, 1972; Hall and Martin, 1974; Springer et al., 1974; Nestorowicz et al., 1985; Formanowski and Meier-Ewert, 1988; Volkman and Golsmith, 1988; Sato et al., 1988). However, the suitability of most of these substances for antiviral therapy is doubtful, because of the high concentration needed to abolish infectivity [13 mg/ml/4–24 h of incubation except for caseinase C which is active at 20 μg/ml/4 h (Reginster, 1965)]. The purified factor of *Echis coloratus* - Echinhibin-1 - is even more useful as it abolishes infectivity at 10 μg/ml (2 h of incubation) or at 1 μg/ml (24 h of incubation).

Most of the antiviral compounds discovered so far inhibit the viral

regulatory gene products, viral proteases and endonucleases (Furman et al., 1986; Vrang et al., 1987; Ngan et al., 1988; Oxford et al., 1989; Tabba et al., 1989; Rooke et al., 1990). This study, of a different and new antiviral agent purified from a snake venom, may represent a new source of effective agents that could prove of major importance for research and clinical management of viral diseases. Future studies should address the specific target of Echinhibin-1 on the virion, in order to reveal the mechanism involved in the process of the inhibition of Sendai virus and examine the possibility of inhibition of other enveloped viruses by Echinhibin-1.

Acknowledgements

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