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Biochemical and Biophysical Research Communications 320 (2004) 190–196
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Antiviral activity against human immunodeficiency virus-1 in vitro by myristoylated-peptide from *Heliothis virescens*

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Received 16 May 2004
Available online 11 June 2004

Abstract

An insect antiviral compound was purified from *Heliothis virescens* larval hemolymph by gel-filtration high pressure liquid chromatography (HPLC) and C-18 reverse-phase HPLC and its structure was determined by mass spectrometry. The antiviral compound is an N-myristoylated-peptide containing six amino acids with calculated molecular weight of 916 Da. The N-terminus contains the fatty acid myristoyl, and the C-terminus contains histidine with two methyl groups giving the histidine a permanent positive charge. The remainder of the compound is essentially non-polar. The structure of the compound corresponds with the 'myristate plus basic' motif expressed by certain viral proteins in their binding to the cytoplasmic side of the plasma membrane to initiate viral assembly and budding from a host cell. The insect antiviral compound may inhibit viral assembly and/or budding of viruses from host cells that could include the human immunodeficiency virus-1 (HIV-1) and herpes simplex virus-1 that use this motif for exit from a host cell. Using the formazan assay, the myristoylated-peptide was effective against HIV-1, with a nine times increase in the viability and protection in vitro of treated CEM-SS cells when compared with infected but untreated control cells.

Insects are subject to many viral infections [1] and have apparently developed broad-spectrum antiviral activity or innate immunity to many DNA and RNA viruses [2,3]. Antiviral mechanisms of insects [4–6] have been poorly understood until more recently with *Heliothis virescens* [2,3]. We found that antiviral activity was present in melanized hemolymph (blood) of tobacco budworm (TBW) insect larvae, *H. virescens* [2,3]. Melanized hemolymph from both vaccinated and unvaccinated TBW larvae was shown to possess antiviral activity against seven DNA and RNA viruses that can infect humans including as described here the human immunodeficiency virus (HIV-1). It was previously determined that the insect melanization reaction [7], activated by phenoloxidase [7,8], provided the antiviral activity [2,3]. Being a natural product, the hemolymph antiviral factor also showed very low cytotoxicity to tissue culture cells [3].

The *Heliothis* inducible cecropins were found to be antibacterial but not antiviral [9,10]. These previous studies indicated that hemolymph antiviral activity was not an inducible response [10] but instead was produced by the insect melanization reaction [2,3]. Insects are known to produce many antibacterial factors in the hemolymph in response to injection of bacteria [11–15]. In contrast, antiviral immunity [5,16,17] has been a poorly understood aspect of insect immunity until clarification that the insect melanization reaction is antiviral [2,3].

The development of new antiviral agents is needed to support medicine. This study describes the discovery of a novel antiviral agent with in vitro antiviral activity against HIV-1 and herpes simplex virus-1 (HSV-1). The primary objectives of the study were to purify and determine the molecular structure and antiviral activity of the insect antiviral compound. The structure of the insect antiviral compound corresponds with the 'myristate plus basic' motif that certain viral proteins use in their binding to the cytoplasmic side of the plasma membrane in order to initiate viral assembly and/or budding from a

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doi:10.1016/j.bbrc.2004.05.137

host cell [18]. The insect antiviral compound discovered here may inhibit this process as a mechanism of action.

Materials and methods

Maintenance of larvae. Heliothis virescens (tobacco budworm) eggs were obtained from the Bioenvironmental Insect Control Laboratory, US Department of Agriculture, Stoneville, MS. After 3–4 days, the eggs hatched. Larvae were reared individually in plastic cups (30 ml) with lids. Each cup contained 10 ml of diet media [19,20]. The cups were held at 25 °C in a natural photoperiod.

Collection and preparation of hemolymph. Hemolymph (blood) was collected with capillary tubes from fifth-instar larvae under ice-chilling conditions [19]. Cell-free hemolymph was obtained by centrifugation at 10,000 rpm for 10 min at 4 °C [19]. Protein concentration of the larval hemolymph was 72 mg/ml. The hemolymph was stored at −80 °C.

The hemolymph was thawed at room temperature and then centrifuged at 10,000 rpm for 10 min at 4 °C to separate the melanin precipitate. The melanin precipitate, after washing once with sterile water, was solubilized in 0.05 M phosphate buffered saline, pH 7.55, with vortexing. Not all of the precipitate dissolved. Following centrifugation, the supernatant was applied to a gel-filtration HPLC column as described below. The solubilized melanin was quantitated using an extinction coefficient equation at 400 nm [21].

High pressure liquid chromatography. The solubilized melanin precipitate was quantitated for melanin and protein (42 mg/ml melanin; 2.6 mg/ml protein). Solubilized products of the insect melanization reaction were analyzed by high pressure liquid chromatography (HPLC) (ISCO Instrumentation, Lincoln, NE) to isolate and purify the hemolymph antiviral factor. Three major and four minor peaks were seen when the solubilized melanin was applied to a low molecular weight Shodex KW-802.5 gel-filtration (separation range 100–50,000 Da) HPLC column (Millipore, Milford, MA) in 0.2 M sodium phosphate buffer, pH 7.0. One milliliter tube fractions of these peaks were collected. Three of the low molecular weight fractions (#12, #15, and #16) showed major anti-HIV-1 activity using the formazan assay (Table 1). One of the three tube fractions (#16) showed the best antiviral activity against HIV-1 and was further purified and separated by reverse-phase HPLC (C-18 column, Rainin Instrument, Woburn, MA) using the experimental conditions described below.

Elution of antiviral retention fractions by C-18 reverse-phase HPLC was performed with a linear gradient of 0–50% acetonitrile in 0.1% trifluoroacetic acid (TFA) over a period of 50 min at a flow rate of 1 ml/min. The organic solvent acetonitrile/water (50/50 v/v) containing 0.1% TFA was used as the final concentration of eluant. Ultraviolet absorption was monitored at 280 nm. The eluted retention fractions were vacuum concentrated (Speed Vac, Savant, Farmingdale, NY) and then dissolved in 0.05 M phosphate buffered saline, pH 7.2. The formazan assay for anti-HIV-1 activity was done on the purified retention fractions obtained by C-18 reverse-phase HPLC.

Anti-HIV-1 data (Table 2) indicated that the major antiviral activity was confined to retention fraction (#22) following C-18 reverse-phase HPLC of tube fraction (#16) obtained by gel-filtration HPLC (Figs. 1 and 2). This retention fraction (#22) from C-18 reverse-phase HPLC that showed the best antiviral activity was then analyzed by mass spectrometry (MALDI-TOF and MALDI-CID) to determine the molecular weight, amino acid sequence, and structure of the insect antiviral compound (Fig. 3).

Biological mass spectrometry. The molecular weight (<1 kDa), amino acid sequence, and structural elucidation of the antiviral compound purified by C-18 reverse-phase HPLC were determined using mass spectrometric methods [22–24]. A matrix-assisted laser desorption/ionization (MALDI) time-of-flight (TOF) and collision-induced dissociation (CID) using Xe (MALDI-TOF and MALDI-CID) mass spectrometer instrument (Voyager RP, Perseptive Biosystems, Framingham, MA) was used to analyze the insect antiviral compound. This instrument can analyze small molecular weight compounds to a sensitivity level of fmol–pmol.

In vitro formazan assay for HIV-1 antiviral activity. A spectrophotometric assay was employed that reflects the extent of inhibition of HIV-1 replication [25]. The source of HIV-1 was from H9 (a CD4+ T-lymphoblastoid cell line) infected cells maintained in RPMI-1640 medium.

In the formazan assay for Table 1, 50 μl CEM-SS CD4+ T-target cells (50,000 cells) in 100 μl RPMI-1640 medium was incubated in wells at 37 °C in 5% CO_2 for 7 days with 50 μl of an antiviral fraction from gel-filtration HPLC and 50 μl of cell-free HIV-1 virus from H9 cells. Then 50 μl of XTT/PMS was added followed by 5 h of incubation and results were read at 450 nm for the amount of formazan product formed. For the controls, phosphate buffered saline, pH 7.2, was used instead of an antiviral fraction.

Table 1
Formazan assay results and indirect fluorescent antibody (FA) test (+1 to +5 fluorescence) results using anti-p24 as the primary antibody

CEM-SS cells and fraction treatment	OD 450 nm	Viability (%)	FA
CEM cells (no HIV-1, no fraction)	1.303	100	0
CEM cells (HIV-1, no fraction)	0.165	12.7	+5
CEM cells (HIV-1, fraction #12)	0.812	62.3	+1
CEM cells (HIV-1, fraction #13)	0.835	64.1	+3
CEM cells (HIV-1, fraction #14)	0.753	57.8	+2
CEM cells (HIV-1, fraction #15)	0.826	63.4	+2
CEM cells (HIV-1, fraction #16)	0.847	65.0	+1

CEM-SS cells were infected with HIV-1 and treated with tube fractions (#12–#16), isolated by gel-filtration HPLC, and then compared with uninfected and infected but untreated control cells for protective effect. The amount of soluble formazan product formed by viable cells was measured at OD 450 nm and the percentages of cell viability then calculated. These were the five fractions showing the greatest anti-HIV-1 activity. Fraction #16 showed a five times increase in protecting CEM-SS cells from HIV-1 infection when compared with the infected but untreated CEM-SS control cells. The formazan fraction values are the means of duplicate determinations, and the controls are the means of triplicate determinations.

Table 2
Formazan assay results measured at 450 nm of CEM-SS cells infected with HIV-1 and treatment with two retention fractions purified by C-18 reverse-phase HPLC and their comparison with uninfected and infected but untreated control cells for protective effect

CEM-SS cells and fraction treatment	OD 450 nm	Viability (%)
CEM cells (no HIV-1, no fraction)	1.793	100
CEM cells (HIV-1, no fraction)	0.136	7.6
CEM cells (HIV-1, fraction #22)	1.184	66.0
CEM cells (HIV-1, fraction #32)	0.622	34.7

These were the two retention fractions (#22, #32) that showed the greatest anti-HIV-1 activity. Tube fraction #16 from gel-filtration HPLC was applied here to a C-18 reverse-phase HPLC column to further purify the anti-HIV-1 compound and obtain the two retention fractions with anti-HIV-1 activity. Retention fraction #22 showed a nine times increase in protecting CEM-SS cells from HIV-1 infection when compared with the infected but untreated CEM-SS control cells. The formazan fraction values are the means of duplicate determinations, and the controls are the means of triplicate determinations.

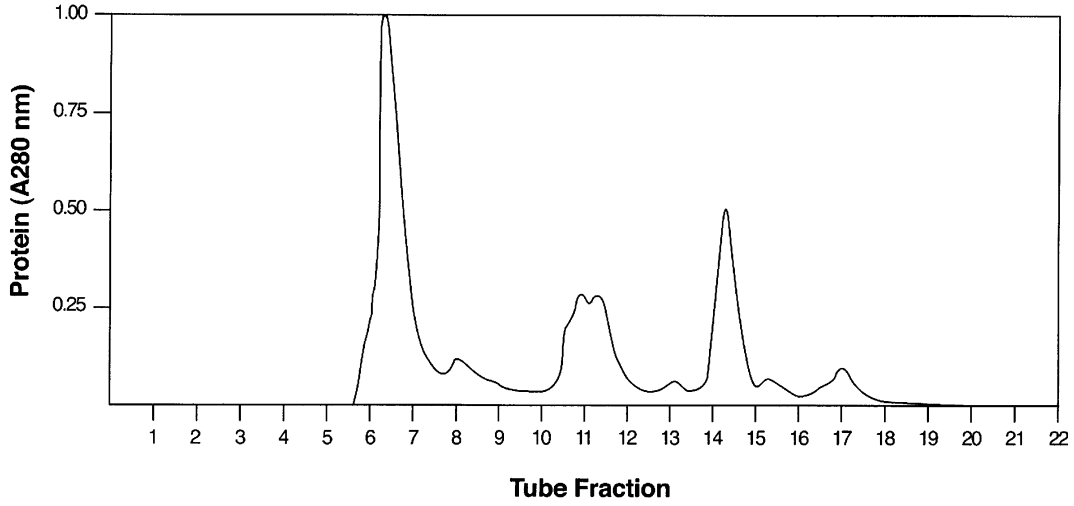

Fig. 1. The solubilized melanin revealed three major and four minor peaks after separation using a Shodex gel-filtration HPLC column (separation range 100–50,000 Da). One milliliter tube fractions were collected and tested for their anti-HIV-1 activity using the formazan assay. Three of the tube fractions (#12, #15, and #16) showed major anti-HIV-1 activity (Table 1).

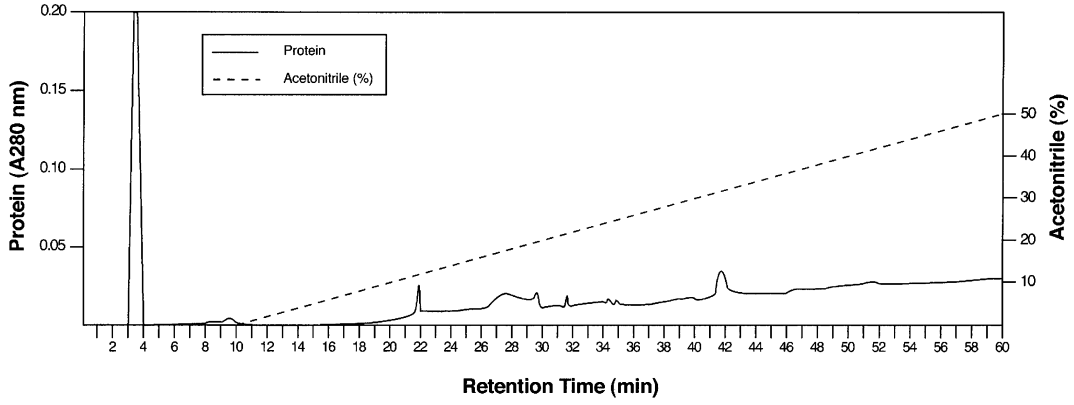

Fig. 2. Tube fraction #16 from gel-filtration HPLC was further purified by C-18 reverse-phase HPLC and retention fractions were tested for anti-HIV-1 activity by the formazan assay to determine the most important antiviral retention fraction. The major anti-HIV-1 activity was confined to retention fraction #22 (Table 2). The molecular structure of the insect antiviral compound was then determined by mass spectrometry of retention fraction #22.

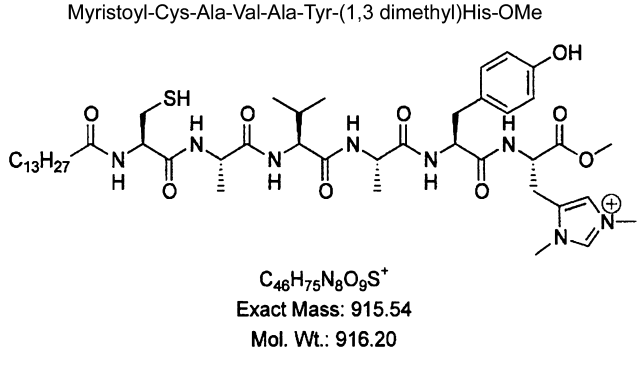

Fig. 3. The structure of the insect antiviral compound was determined by mass spectrometry of retention fraction #22 from C-18 reverse-phase HPLC (Fig. 2). The antiviral compound is composed of six amino acids and one fatty acid. At the N-terminus of the myristoylated-peptide is found the 14-carbon fatty acid myristoyl or myristate. The positively charged C-terminus contains a histidine with two methyl groups (L-histidine, 1,3-dimethyl, methyl ester) that has not before been found in nature. The calculated molecular weight of the antiviral compound is 916 Da.

In the formazan assay for Table 2, 50 μl CEM-SS CD4+ T-target cells (50,000 cells) in 50 μl RPMI-1640 medium were incubated in wells at 37 °C in 5% CO_2 for 7 days with 125 μl of an antiviral fraction from C-18 reverse-phase HPLC and 25 μl of cell-free HIV-1 virus from H9 cells. Then 50 μl XTT/PMS were added followed by 4 h of incubation and results read at 450 nm for the amount of formazan product formed. For the controls, phosphate buffered saline, pH 7.2, was used instead of an antiviral fraction.

In the formazan assay, a soluble colored XTT formazan product is formed by viable cells after cell incorporation of XTT tetrazolium/PMS (*N*-methylphenazonium methosulfate). Since only viable CEM-SS cells (grown in 96-well microtiter plates) develop the formazan color, the amount of formazan product produced can be measured spectrophotometrically at 450 nm using an ELISA Bio-Tek Instrument EL 210 (Bio-Tek Instruments, Burlington, VT) microplate reader. The antiretroviral effect of the gel-filtration HPLC and C-18 reverse-phase HPLC purified insect antiviral compound for protecting CEM-SS target cells against HIV-1 was therefore determined. Formazan assay determinations of the antiviral fractions were done in duplicate and in triplicate for the controls in Tables 1 and 2.

Indirect fluorescent antibody procedure for HIV-1 p24 core antigen. An indirect fluorescent antibody (FA) test was used to determine the

number of CEM-SS cells infected with HIV-1 after their treatment with an antiviral fraction [26]. Ten microliter containing 10,000 CEM-SS cells (infected with HIV-1 for 5 days and treatment with 50 μl of an antiviral tube fraction) from a microtiter well were applied to a glass microscope slide and dried overnight at room temperature. The CEM-SS cells were then fixed in absolute methanol for 40 min. Then a 1:10 dilution of sheep IgG to p24 core antigen (Sigma, St. Louis, MO) was added to the glass slide and incubated for 30 min. After washing the slide in phosphate buffered saline, pH 7.2, a 1:50 dilution of fluorescein-conjugated goat-antisheep IgG was added and incubated for 30 min. The CEM-SS cells were then viewed with a Zeiss fluorescent microscope for determination of +1 to +5 fluorescence. Test determinations were done in duplicate. This procedure indicated the degree of HIV-1 infection of CEM-SS cells after their treatment with an antiviral tube fraction (#12–#16) from gel-filtration HPLC and comparison with the uninfected and infected control cells (Table 1).

Antiviral assay against herpes simplex virus-1 in tissue culture. The antiviral compound in solubilized melanin was tested for antiviral activity at a 1:100 dilution against herpes simplex virus-1 (HSV-1) in tissue culture wells. Antiviral activity was determined using an assay done with MRC-5 human fibroblast cells in Dulbecco's modified Eagle's medium that demonstrated viral cytopathic effect [3,10,27]. The antiviral compound was added to the cell monolayer 24 h before infection with HSV-1. These results were compared with those of phosphate buffered saline, pH 7.2, being added, instead of the antiviral compound, before infection of tissue culture cells with HSV-1. Three days of incubation at 37 °C were done before adding XTT/PMS followed by an additional 4 h of incubation. The results were read at 450 nm for the amount of formazan product formed by viable cells. Assay determinations were done in duplicate.

Tube fractions (#15, #16) from gel-filtration HPLC (Fig. 1) were tested for prevention of the characteristic viral cytopathic effect (CPE). Five hundred microliters of Dulbecco's modified Eagle's medium was added to wells containing a monolayer of MRC-5 human fibroblast cells. One hundred microliters of tube fractions #15 and #16 or 100 μl of phosphate buffered saline, pH 7.2, was added to separate wells followed by incubation for 24 h at 37 °C in 5% CO_2. Then 50 μl of HSV-1 virus was added and incubated for 24 h at 37 °C in 5% CO_2. The CPE was observed as +1 to +5 for cell damage and lysis. The lowest CPE (+1 to +2) indicated 20–30% cell damage and lysis and indicated antiviral activity. The highest CPE (+5) indicated 90% or more cell damage and lysis and no antiviral activity as seen with the phosphate buffered saline control. The fractions were also heated for 1 h at 56 °C to see if this temperature would affect the CPE results. Assay determinations were done in duplicate.

Protein determination. Hemolymph protein was determined by a modification of the Lowry method [28] using the BCA protein assay (Pierce Chem., Rockford, IL). Bovine serum albumin was used as the protein standard.

Results

The solubilized melanin revealed three major and four minor peaks after initial separation using a low molecular weight Shodex gel-filtration HPLC column (separation range 100–50,000 Da) (Fig. 1). One milliliter tube fractions were collected (fractions #6–#18) and tested for their anti-HIV-1 activity using the formazan assay. At least three of these tube fractions (#12, #15, and #16) from gel-filtration HPLC (Fig. 1) showed major anti-HIV-1 activity (Table 1). One of these tube fractions (#16) was a small peak that showed the best anti-HIV-1 activity (Table 1). Tube fraction #16 showed a five times increase (65% vs. 12.7%) in protecting CEM-SS cells from HIV-1 infection when compared with the infected but untreated CEM-SS control cells (Table 1). Tube fraction #16 from gel-filtration HPLC was then further purified by C-18 reverse-phase HPLC, and the several retention fractions obtained were tested for their anti-HIV-1 activity by the formazan assay. The data indicate that the major antiviral activity was confined to HPLC retention fraction (#22) obtained from gel-filtration HPLC tube fraction #16 (Table 2; Fig. 2).

The purified insect antiviral compound showed a nine times increase (66% vs. 7.6%) in the viability and protection of CEM-SS cells from HIV-1 infection when compared with the infected but untreated control cells (Table 2). Retention fraction (#22) from C-18 reverse-phase HPLC was analyzed by mass spectrometry (MALDI-TOF and MALDI-CID) to determine the molecular weight and structure of the antiviral compound (Fig. 3). The insect antiviral factor is a myristoylated-peptide that has a calculated molecular weight of 916 Da (Fig. 3) and is likely an organic metabolite of melanin biosynthesis [3,7,8].

The insect antiviral compound (retention fraction #22 from C-18 reverse-phase HPLC of gel-filtration HPLC tube fraction #16) (Tables 1 and 2; Figs. 1 and 2) was analyzed by mass spectrometry and is composed of six amino acids and one fatty acid (Fig. 3). The C-terminus of the myristoylated-peptide contains the amino acid L-histidine, 1,3-dimethyl, methyl ester. The two methyl groups of the histidine ring give the amino acid a permanent positive charge. The histidine structure with two methyl groups is an amino acid that has not before been found in nature. At the N-terminus of the myristoylated-peptide is found the 14-carbon fatty acid myristoyl or myristate. Except for the C-terminal amino acid histidine which has a permanent positive charge, the rest of the compound is essentially non-polar with the five amino acids that are either non-polar or have an uncharged polar group and the myristate fatty acid (Fig. 3). This structure corresponds then with the 'myristate plus basic' motif expressed by certain viral proteins to initiate viral budding from a host cell [18].

The solubilized melanin was effective in preventing infection of MRC-5 human fibroblast cells with HSV-1. A 53% viability of MRC-5 cells was observed after infection and incubation of cells with HSV-1 and comparison with uninfected MRC-5 control cells. The mean of optical density readings at 450 nm for the formazan product produced by viable cells was 1.3 OD for uninfected cells and 0.69 OD for infected cells.

The tube fractions (#15, #16) from gel-filtration HPLC (Fig. 1) gave +1/+2 readings for prevention of CPE by HSV-1 infection when compared with +5 CPE readings for the phosphate buffered saline control. Heating the fractions for 1 h at 56 °C did not affect the +1/+2 CPE results.

Discussion

The initial discovery of a novel broad-spectrum antiviral agent was accomplished by previous insect immunity research [2,3]. The insect antiviral factor previously discovered has been purified, its structure determined (Fig. 3), and possibly could be used as an antiviral agent against human viral infections like HIV-1 and HSV. The purification and characterization of an antiviral compound produced by an insect larva is an important discovery of potential medical importance.

It was previously determined that inactivation of viruses by the insect antiviral factor was a result of the hemolymph melanization reaction [3,8]. Previous data indicated that broad-spectrum antiviral activity was produced by *H. virescens* hemolymph (blood) to the six DNA and RNA viruses tested [3], which now also includes HIV-1 (Tables 1 and 2). All of these viruses are enveloped viruses except one [27]. Being a natural product, the insect antiviral factor also showed very low cytotoxicity to cells [3].

In the previous study [3], the importance of the insect hemolymph antiviral activity could be seen by calculating the *R* (therapeutic index) values or the measure of cytotoxicity (mg/ml) divided by the antiviral activity (mg/ml) [27]. An *R* value of 10 is needed in order for antiviral activity to be effective in animals. The *R* value of the hemolymph antiviral factor for both of the DNA viruses (HSV-1 and HSV-2) was 110 which is excellent for an antiviral agent and is 11 times greater than what is needed to be effective in animals. In fact, the *R* value of 110 for the hemolymph antiviral factor was greater than the *R* value of 63 for the antiviral standard (Acyclovir) that was used. Acyclovir is an antiviral drug used to treat herpesvirus infections. The hemolymph antiviral factor was also much more effective in antiviral activity against the vesicular stomatitis virus (VSV), an RNA virus, than was the ribavirin standard. The *R* value of 45 for antiviral activity against VSV was over four times what is needed to be effective in animals. The hemolymph factor was also antiviral against parainfluenza-3 (*R* value of 16), Coxsackie B3 (*R* value of 20), and Sindbis (*R* value of 20) viruses.

Certain N-myristoylated proteins can be localized to intracellular membranes and the plasma membrane [18,29]. The antiviral compound (Fig. 3), which corresponds to a 'myristate plus basic' motif, may be directed to the inner leaflet of the plasma membrane thus blocking virus assembly and/or budding and exit of viruses from a host cell [18]. The antiviral factor is of low molecular weight (<1 kDa) and apparently able to cross the plasma membrane where it may inactivate then HIV-1 assembly and/or budding from infected cells.

N-myristoylation of proteins enhances their internal membrane-binding ability [18]. Retroviral and lentiviral Gag proteins require myristoylation in order to bind to the plasma membrane and thus mediate virion formation and assembly [18]. Myristate or a cluster of the basic residues inserts hydrophobically into the lipid bilayer, and basic amino acids then form electrostatic interactions with head groups of acidic phospholipids. This constitutes the 'myristate plus basic' motif that certain viral proteins use for binding internally to plasma membranes. The insect antiviral compound identified here (Fig. 3) resembles a 'myristate plus basic' motif that may bind internally to the plasma membrane.

The HIV-1 Gag protein contains myristate of the 'myristate plus basic' motif at its N-terminus that is necessary for binding to the cytoplasmic side of the plasma membrane [18]. Virion cores are assembled in the cytosol and myristoylation of Gag protein is required for intracellular transport to the plasma membrane [30]. The HIV-1 Gag protein then is directed to the plasma membrane by a 'myristate plus basic' motif [31]. The HIV-1 Gag proteins make up the viral nucleocapsid [32]. The Gag gene codes for internal structural proteins like the p24 core protein. The Gag gene has the ability to direct the formation of virus-like particles. When the Gag gene is non-functional, then retroviruses like HIV-1 lose their capacity for budding from a host cell [32]. The insect antiviral compound purified here (Fig. 3) may inhibit or block the binding of the Gag protein to the plasma membrane and thus prevent assembly and/or budding of retrovirus from the host cell. This possibly may account for its antiviral activity (Tables 1 and 2).

The antiviral factor was also effective by the indirect fluorescent antibody technique using anti-p24 antibody (Table 1). The fluorescence seen with HIV-1 infected CEM-SS cells treated with the insect antiviral factor was compared with that seen with the infected but untreated CEM-SS control cells that gave a +5 fluorescence (Table 1). Tube fraction #16, isolated by gel-filtration HPLC, showed a low +1 fluorescence for HIV-1 infection and also gave 65% viability for CEM-SS cells (Table 1). Retention fraction #22 was purified by C-18 reverse-phase HPLC from tube fraction #16 that was obtained by gel-filtration HPLC (Figs. 1 and 2). Retention fraction #22 protected CEM-SS cells from HIV-1 infection by nine times when compared for viability with the infected but untreated CEM-SS control cells (66% vs. 7.6% viability of CEM-SS cells) (Table 2).

The low molecular weight insect antiviral factor (916 Da), having anti-HIV-1 activity (Tables 1 and 2), was purified by gel-filtration HPLC and C-18 reverse-phase HPLC (Figs. 1 and 2). Mass spectrometry analysis to determine its structure and composition was then done on retention fraction #22 from C-18 reverse-phase HPLC (Fig. 3). The structure of the insect antiviral compound corresponds with the 'myristate plus basic' motif expressed by certain viral proteins in their internal binding to the membrane lipid bilayer [18]. The insect

antiviral compound resembles a 'myristate plus basic' motif, developed by evolutionary design and tested over millions of years for efficacy in nature's laboratory. The insect antiviral compound has myristate, a 14-carbon saturated fatty acid, at one end (N-myristoylated) and is positively charged (basic) at the other end with the permanently positive-charged histidine containing two methyl groups (Fig. 3). This version of histidine with two methyl groups has not before been found in nature and would help facilitate the membrane-binding of myristate internally to the plasma membrane. The 'myristate plus basic' motif is known to play an important role in the plasma membrane-binding of certain viral proteins that initiate viral budding from a host cell [18]. Most enveloped viruses bud from the plasma membrane. A probable mechanism of action then of the insect antiviral compound may be that of an exit inhibitor that is effective against several different enveloped viruses [3] that use this motif for budding and exit from host cells [18,27]. It is plausible that the insect antiviral compound is therefore able to block or inhibit viruses like HIV-1 and HSV that use this motif for exit from a host cell [18].

The calculated molecular weight of the antiviral compound is 916 Da (Fig. 3). The small molecular weight and non-polar nature of the insect antiviral compound should enhance its ability to cross the plasma membrane, in addition to its binding to the pocket for the 'myristate plus basic' motif found in the plasma membrane. The consequence would be blocking or inhibiting viral assembly and/or budding from the host cell and possibly accounting for its antiviral activity (Tables 1 and 2).

The novel compound isolated here may also be responsible for the broad-spectrum antiviral activity previously observed against the six DNA and RNA viruses tested [3]. The insect antiviral compound purified here (Fig. 3) was also effective against HIV-1 (Tables 1 and 2) and HSV-1.

Millions of people are infected world-wide with HIV-1 [33]. Due to the world-wide occurrence of HIV-1 and AIDS, many attempts are being made to develop drugs that stop replication or interrupt a step in the process of virion assembly [34]. Resistance has been developed to the reverse transcriptase inhibitors and protease inhibitors which currently are the primary drugs used to treat AIDS [34,35]. The insect antiviral compound purified here might prove effective against HIV-1 in the treatment of AIDS by blocking the assembly and/or budding of HIV-1 from infected T-lymphocytes.

Acknowledgments

Thanks are due to the following individuals: Dr. William Lewis, Center for Biotechnology, St. Jude Children's Research Hospital, Memphis, TN, for doing mass spectrometry structural analysis; and Mr. Jesse Ingels, Department of Medicine, University of Tennessee Health Science Center, Memphis, TN, for assistance in doing anti-HIV-1 and anti-HSV-1 activity testing.

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