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ORIGINAL ARTICLE

INHIBITION OF HIV ENTRY BY CARBOHYDRATE BINDING PROTEIN ISOLATED FROM MARINE CRAB *Portunus pelagicus* (LINNEUS, 1775)

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ABSTRACT

Lectins are carbohydrate binding proteins of non- immunoglobulin nature. They are involved in many biological processes like recognition and binding of carbohydrates, host-pathogen interaction, antiviral, antifungal and antibacterial activities. Some of the lectins isolated from algae, fungi, plants and animals exhibit significant activity against human immune-deficiency virus (HIV) and other enveloped virusesHowever, lectins derived from marine organisms have a rich source of natural antiviral protein. In the present investigation, lectins isolated from thehaemolymph of marine crab *Portunuspelagicus*was tested against HIV samples. The positive samples show the reactive values which resulting from the samples binding activity with the monoclonal antibodies found in the solid phase. The negative samples show the non-reactive values which resulting from the non- binding activity. The lectins adding samples show lesser values than the non lectins samples. This indicates that PPL inhibits HIV- by binding to the glycosylated viral envelope and blocking cellular entry and multiplication.

Keywords: Carbohydrate binding protein, *Portunus pelagicus*

1.INTRODUCTION

Lectins are carbohydrate binding proteins of non-immunoglobulin nature. They are involved in many biological processes like recognition and binding of carbohydrates, host-pathogen interaction, antiviral, antifungal and antibacterial activities. They are naturally found in wide variety of organisms like prokaryotes, algae, fungi, higher plants, invertebrates and vertebrates. Some of the lectins isolated from algae, fungi, plants and animals exhibit significant activity against human immune- deficiency virus (HIV) and other enveloped viruses, which makes develop a novel antiviral drugs (De Chercq, 2005). However, lectins derived from marine organisms have a rich source of natural antiviral protein (Tziveleka et al., 2003). The surface of retroviruses such as HIV and many other enveloped viruses are covered by virally encoded glycoprotein gp120 and gp41. Lectins can their ability to bind mannose containing oligosaccharides present on the surface of viral envelope glycoproteins has been described earlier by Hansen et al., (1989) and Charan et al., (2000). They were able to inhibit fusion of HIV –infected cells with CD_4 cells by a carbohydrate specific interaction with the HIV-infected cells.

The human immunodeficiency virus (HIV) is an enveloped virus that causes the acquired immunodeficiency syndrome (AIDS). In human the condition is progressively failure of the immune system, it infects T-helper cells, macrophages and dendritic cells as a result low level of CD_4 cells through a number of mechanisms. Cyanovirin-W and griffithsin are examples of lectins that can inhibit HIV and other viruses (Boyd et al., 1997, and Mori et al., 2005). Different lectins have different anti HIV mechanisms. The treatment of AIDS with lectins is being investigated in many studies. Recently, lectins from the polychaete marine worm chaetopterus*Variopedantus*inhibited cytopathic effect induced by HIV-1 and the production of viral p_{24} antigen (Wang et al., 2005).

Lectins of different carbohydrate specificities are able to promote growth inhibition or death of bacteria, fungi and virus. Lectins are potential drugs for treatment of AIDS.

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Lectins (D-mannose-specific) can able to inhibit fusion of HIV infected cells with CD4 cells by a carbohydrate specific interaction with the HIV infected cells (Hansen et al., 1989). The antibacterial, antifungal and antiviral activities of lectins have been reported (Boyel et al., 1997 and Singh et al., 2014). Lectins have become the focus of intense interest for biologists and in particular for the research and application in agriculture and medicine (Movafagh et al., 2013).In the present investigation, lectins isolated from the marine crab *Portunuspelagicus*inhibit by binding with glycoprotein envelop of HIV and prevent entry to cells andinfection and multiplication of HIV.

2.MATERIALS AND METHODS

Collection of experiment animal

The marine crab*Portunuspelagicus*were obtained from the sea shore area along the Managudi estuary region at Kanyakumari district. They are transported to laboratory with care and maintained in cement tank with aerated natural seawater until further use. For sample collection, walking legs of the crab was cutting with a fine sterile scissor and collect approximately 3-4 ml haemolymph in a clean sterile plastic vessel, which contains sodium citrate buffer, pH 4.6 and equal volume of physiological saline (0.85% NaCl, w/v). serum was separated by centrifuge the sample at 10,000 rpm at 4^{o}Cfor 10 minutes. The haemocytes were precipitated and the supernatant were collected by aspirating and stored at 4^{o}C until used. The protein content of the haemolymph and other fractions was estimated by the method of Bradford using BSA as the standard (Brandford, 1976).

Enzyme Linked Immuno Lectins Sorbant Assay (ELILSA)

The affinity of PPL for different glycoproteins was studied by ELILSA. The binding of fixed amount of PPL to varying amounts of glycoproteins coated on the wells of microtiter plates (BeneSphera^{TM}HIV Advance) was checked. Samples with absorbance greater than or equal to the cut off value are considered reactive to anti-HIV and HIV antigen (positive). Samples lesser than the cut off value are considered non-reactive to anti-HIV and HIV antigen (negative).
BeneSphera^{TM} HIV Advance is a fourth generation Elisa test for the qualitative determination of antibodies to HIV-I (group O and M), HIV-2 and P24 Antigen of HIV-1 in human serum or plasma.

Specimen collection and handling;

Serum and plasma (citrate or EDTA) samples may be stored for up to 7 days at 2-8^{0}C or at least 6 months at frozen (-20 to -70^{o}C). Samples should not be repeatedly frozen and thawed. Heat inactivated serum or plasma specimens may cause false HIV reactive results. Do not use sodium azide as preservative because it inactivates horseradish peroxidase.
Handle specimens carefully for capability of infection of unknown viruses or HIV.
Do not allow substrate to come in contact with skin.
Avoid microbial contamination of reagents when opening and removing aliquots from the vials.

Assay procedure:

1. Take the required numbers of strips and fix them to frame.
2. Take equal amount of sample in all wells. Pipette out 100 µl of negative control into each well of 1A to1B, 100 µl of antibody positive control into well of 1C, 100 µl of antigen positive control into well of 1D. (Take care not to mix or splash contents out of wells while using microplate shaker).
3. Incubate at 37± 1^{o}C for 60 minutes after sealing the plate with cover sealer.
4. Before the last 10 minutes of 1st incubation, make a 1:51 dilution of conjugate with conjugate diluent.
5. Aspirate the contents from all the wells and wash each one 5 times with 300 µl of diluted washing solution.
6. Invert the plate and tap it on absorbent paper to remove the remaining washing solution and then, pipette out 100 µl of prepared diluted conjugate into each well.
7. Incubate the plate at 37 ± 1^{o}C for 30 minutes after sealing it with plate sealer.
8. Before the last 5 to 10 minutes of second incubation, make a 1: 101 dilution of substrate with substrate buffer.
9. Aspirate the contents from each of the wells and wash each one 5 times with 300 µl of diluted washing solution.
10. Invert the plate and tap it on absorbent paper to remove the remaining washing solution and then, pipette out 100 µl of prepared substrate into each well and incubate at controlled room temperature (23 ± 2^{o}C) for 30 minutes. Avoid exposure to light.
11. Pipette out 100 µl of stop solution into each well and tap the plate gently to homogenize the colouring materials.
12. Read the absorbance at 450 nm (reference wavelength at 620 nm) against an air blank within 30 minutes after addition of stop solution.

Quality control:

- The average absorbance (PCx) of the positive controls should be greater than or equal to 1.
- The average absorbance (NCx) of the negative control should be less than or equal to 0.1 and greater than -0.005.

If the results are outside the above range, the test should be conducted again.

Calculation of the cut off value:

Calculate the Negative Control mean (NCx) = (a_1 + a_2 + …….a_n/ n)
Where, a_1 = first absorbance value, a_2 = second absorbance value, n = total number of absorbance.
Cut off value = NCx + 0.200 = 0.244

Interpretation:

Samples with absorbance greater than or equal to the cut off value (0.244) are considered reactive to anti-HIV and HIV antigen. Samples with absorbance less than the cut off value are considered non -reactive to anti-HIV and HIV antigen.

If the samples are considered reactive, the test should be conducted two more times. In case the retests show non-reactive results the samples are considered negative, and on the other hand, if one of the retest shows reactive result the samples are considered positive.

3.RESULTS

In the present investigation the lectins isolated from the haemolymph of marine crab *Portunuspelagicus*is tested antiviral activity by enzyme linked immune and lectins sorbent assay (ELILSA) method, the samples reactivity of the test OD values obtained was shown in the table (1). The average absorbance of positive antigen control (PCx) is 1.479 and the positive antibody control is 1.612. The average absorbance of the negative control is 0.014. The calculated cut off value is 0.214. The five positive samples before adding to lectins OD values are 0.425, 0.524, 0.455, 0.685, respectively and after adding to lectins OD values are0.346, 0.475, 0.407, 0.452 and 0.595 respectively. The negative samples before adding lectins shows 0.023, 0.018, 0.026, 0.019 and 0.022 respectively and after adding to lectins shows 0.021, 0.017, 0.024, 0.018 and 0.021 respectively.

4.DISCUSSION

Lectins are carbohydrate binding proteins of non-immunoglobulin nature. They are capable of recognition of reversible binding to complicated glycoconjugates moieties without altering the covalent structure of any of the recognized glycosyl ligands. The human immunodeficiency virus (HIV) type -1 is an enveloped virus that causes the acquired immunodeficiency syndrome (AIDS). It infects viral cells such as T- helper cells, macrophages and dendritic cells. This infection leads to low levels of CD4+ through a number of mechanisms. Also apoptosis of uninfected cells, direct viral killing of infected cells and killing of infected CD4+ cell by cytotoxic lymphocytes. The surface of retroviruses and other enveloped viruses are covered by virally encoded glycoproteins. Glycoproteins gp_{120} and gp_{41} present on the HIV envelope are heavily glycosylated with 50% of glycans. The antiviral activity of lectins appears to depend on their ability to bind mannose containing oligosaccharids present on the surface of viral enveloped glycoproteins.

Agents that specifically and strongly interact with the glycans may disturb interaction between the proteins of the viral envelope and the cells of the host(Balzarini J. 2006). Sugar binding proteins can crosslink glycans on the viral surface and prevent further interaction with the co-receptors (Sacchettini et al., 2001). Majority of the recent antiviral therapeutics, that acts through inhibition of the viral life cycle. In the present investigation the positive samples shows the reactive values which resulting from the samples binding activity with the monoclonal antibodies found in the solid phase. The negative samples show the non- reactive values which resulting from the non- binding activity. The lectins adding samples show lesser values than the non lectins samples. This indicates that PPL inhibits HIV- by binding to the glycosylated viral envelope and blocking cellular entry and multiplication.

Various lectins have potential microbicides activity to prevent HIV transmission. Michael et al., (2010), isolated lectins (BanLec) from bananas *Musa acuminata* which is a potent inhibitor of HIV replication. The carbohydrate binding red algal lectins KAA-2 from *Kappaphgcusalvarezii*was studed by Sato et al., (2011). He tested the anti- influenza virus activity of KAA-2 against various strains of influenza virus. Haruo Tanaka et al., (2009), isolated lectins actinohivin (AH) from an actinomycetehave potent anti-HIV activity by binding to high mannose type glycans (HMTGs) of gp120, an enveloped glycoprotein of HIV. In the present investigation, the lectins isolated from the crab *Portunuspelagicus*a carbohydrate binding protein binding to HIV could prevent the initial step in viral infection by blocking cellular entry.They binding to the glycosylated viral envelop is a safe microbicide to help prevent HIV transmission.

Enzyme linked immuno and lectin sorbent assay(elilsa)method

Sample	Negative contol-1	Negative control-2	Antigen positive control	Antibody positive control	PS1	PS2	PS3	PS4	PS5	NS1	NS2	NS3	NS4	NS5
before adding to lectins OD value	0.14	0.14	1.479	1.612	0.445	0.524	0.455	0.578	0.685	0.23	0.018	0.026	0.019	0.022
After adding to lectins OD value	0.14	0.14			0.346	0.475	0.407	0.452	0.595	0.021	0.017	0.024	0.018	0.021

PS- positive sample, NS- negative sample, NR- non- reactive, R- reactive

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