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Characterization of Sugar Binding Lectins Isolated from Different Parts of *Eudrilus Eugeniae*

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Abstract

Lectins occur widely in animal and plant kingdom. In animals there is wide range of reports of presence of lectin in vertebrates and invertebrates, but their function is not fully understood in invertebrates. It may either have a role in the developmental stages or in the defense mechanism by providing immunity. In earthworm species like *Eudrilus eugeniae* presence of lectin like proteins, were reported. The aim of this study is to isolate the sugar binding lectin like proteins from Coelomocytes (CC), muscles (MC), gut/whole body (WE) & vermicompost (VC) of earthworms and characterize them. Partially purified lectins were estimated for their protein content by Biuret method and it was observed that lectins isolated from CC have more protein content of 0.178 mg/ml as compared to any other source. Their sugar binding specificity was checked by DNS method and it was observed that CC lectins and VC lectins have more affinity for glucose (CC glu & VC glu) while MC lectin and WE lectins have more affinity for galactose (MC gal & WE gal). The molecular weight was found to be 26 KDa checked by SDS-PAGE. The lectins were then tested for their hem agglutination property for human blood types A^{+ve}, B^{+ve}, AB^{+ve} & O^{+ve} and they showed no agglutination, Later antimicrobial property at various concentration of lectin sample was checked for different pathogenic bacteria and fungus and it was observed that *Escherichia coli,* showed no zone of inhibition, *Bacillus subtilis,* with MC gal showed 2.4 cm zone of inhibition, and *Staphylococcus aureus* where both CC gal and VC gal showed maximum zone of inhibition of 2.6 cm at 200 µl of 1mg/ml lectin in all, fungus like *Aspergillus niger* showed 1.0 cm zone of inhibition with VC gal, *Penicillium chrysogenum* showed 1.5 cm zone of inhibition with CC gal at 200 µl of 1mg/ml lectin respectively and *Fusarium oxysporum* showed no zone of inhibition. The different lectins were then assessed for their mitogenic activity on vertebrate (chicken liver cells) and invertebrate cells (earthworm cells). The cells were cultured in HBSS media and three different concentrations i.e; 0.1mg/ml. 0.01mg/ml & 0.001mg/ml of lectins from different sources were added to the cells along with control. It was observed that invertebrates showed mitogenecity or proliferation of 83.6% at 0.1mg/ml of lectin from WE that is with more concentration of lectin more proliferation was observed while vertebrates showed inhibitory effect with lectin that is with more concentration of lectin less proliferation was observed.

Keywords: lectin, DNS, SDS-PAGE, HBSS, mitogenecity, CC, MC, VC, WE

Introduction

Lectins are proteins that occur widely in animal and plant kingdom (Dodd and Drickamer 2001), but their function is not fully understood. It may either have a role in the developmental stages or in the defense mechanism by providing immunity (Kilpatrick 2001). In earthworm species

like *Eudrilus eugeniae* presence of lectin like proteins, which are sugar binding proteins in various location were reported (Licata *et al* 2002).

The objective of the research work is isolation and assessment of partially purified proteins from coelomic fluid, muscles, gut & vermicompost of earthworms for their specificity for binding to monosaccahrides like glucose and galactose. They will be tested for their antimicrobial properties against selected human pathogens and *in vitro* studies for mitogenic effect on vertebrate and invertebrate cells. This will enable to understand their potential use in future pharmaceuticals.

Materials and methods: *Eudrilus eugeniae* cultured in kitchen waste and leaves in 3:1 ratio for around 1-2 months. Adult earthworms were used for lectin isolation from different parts. Following are the steps in this investigation;

Lectin isolation & purification: coelomocytes (CC) were isolated by cold shock method, and were cultured by incubating in CO_2 incubator for one week in HBSS medium. Muscle cells (MC) were cut in fine pieces and also cultured in CO_2 incubator for one week in HBSS medium, after one week Coelomocytes and muscle cells were trypsined and pelleted and then lysed with 1% SDS and 0.1mM EDTA to release lectin. They were incubated separately with 20 mM glucose and 20 mM galactose in EDTA-MEPBS buffer overnight, Similarly whole body (WE) of earthworms fed on filter paper for 48 hrs were homogenized and cells were pelleted to release lectin with same procedure as above. Vermicompost (VC) was added with ME-PBS buffer to collect supernatant and lectin was isolated. Later all the different lectins were purified by dialysis method using appropriate membrane.

Protein estimation: The concentration of all different proteins was achieved by Biuret method taking BSA as positive control. (Ferdinand Rose, 1833)

Estimation of Sugar binding specificity: The sugar binding specificity can be determined by DNS method where the colour intensity will give the appropriate result. Specific sugars will go and bind to the lectin proteins leaving DNS free which gives more colour. Hence less color intensity less sugar is present (Miller, 1959).

SDS-PAGE: It was done to determine molecular weight of different lectin proteins isolated.

Agglutination properties: Agglutination property of the lectins against human blood type (A+ve, B+ve, AB+ve & O+ve) was checked to find out the affinity between Antigens of proteins and receptors on RBC. This was done by adding one drop of each blood type with one drop of purified 1mg/ml of different lectin samples separately.

Antibacterial and antifungal effect: It was done to determine whether the isolated lectins have any specificity & lytic activity for sugar moieties present on pathogens. The test was performed by agar gel diffusion method taking 50µl, 100 µl, 150 µl & 200 µl of 1mg/ml of lectin samples isolated from different parts of the earthworm on bacteria like *Escherichia coli, Bacillus subtilis* and *Staphylococcus aureus* and on fungus like *Aspergillus niger, Penicillium notatum* and *Fusarium oxysporum.*

Mitogenic activity: Mitogenic activity or proliferation activity of all lectins on vertebrate (chicken liver cells in HBSS media) and invertebrate cells (earthworm muscle cells) was checked to find whether the lectins added can act as growth factor for the developing cell (Imanishi *et al* 1981) (Hrzenjak *et al* 1993). The cells were cultured in HBSS media and three different concentrations i.e; 0.1mg/ml. 0.01mg/ml & 0.001mg/ml of lectins from different sources were added to the cells along with control for one week in CO_2 incubator.

Results and Discussion

Protein estimation assay showed that all the lectins isolated from different parts that is from coelomocytes, muscle cells, whole earthworm body and vermicompost of the earthworm *Eudrilus eugeniae* showed protein content in it which was estimated by Biuret method taking BSA as control at 540 nm.. The lectins isolated from Coelomocytes with affinity for glucose (CC glu) showed maximum protein content of 1.78mg/ml.

Sugar binding assay with DNS was done to find out the specificity of the lectins for a particular sugar. All the lectins showed affinity for sugars glucose and galactose but Coelomocytes and Vermicompost showed greater affinity for glucose while muscle cell and whole earthworm body showed more affinity for galactose. When sugar and lectin bind together, DNS will be left free and hence show less absorbance reading and less colour intensity.

The molecular weight of the four different lectins was determined by SDS-PAGE, stained with silver staining method and was found to be approximately 26 KDa for all lectins.

Hemagglutination was checked to see whether the lectins

have any coagulating effect on the human blood, and it was observed that lectins show no agglutination with the blood suggesting that the lectin isolated from the earthworm has no Ab against the Rh antigen of human blood. Hence it has anti-coagglutant property (Karel and Franklin 1946) and therefore can be further tested as an anti coagulant.

Antimicrobial activity of different lectins was checked on three different pathogenic bacteria and fungus and it was observed that lectins do have antibacterial and antifungal effect. *Bacillus subtilis* showed 2.4 cm zone of inhibition with lectin isolated from muscle cells having affinity for galactose, and *Staphylococcus aureus* showed maximum zone of inhibition of 2.6 cm at 200 µl of 1mg/ml of lectin isolated from coelomocytes having affinity for galactose and lectin from vermicompost with affinity for galactose respectively. *Escherichia coli,* showed no zone of inhibition, while *Aspergillus niger* showed 1.0 cm zone of inhibition with lectin from vermicompost with affinity for galactose, *Penicillium chrysogenum* showed 1.5 cm zone of inhibition with lectins from Coelomocyte having affinity for galactose at 200 µl of 1mg/ml lectin respectively and *Fusarium oxysporum* showed no zone of inhibition. Antimicrobial effect shows that the lectins may bind to the sugar moieties present on the surface of the bacterial cell and lyse it (Liao *et al* 2003) (cooper *et al* 1995).

The different lectins were then assessed for their mitogenic activity on vertebrate (chicken liver cells) and invertebrate cells (earthworm cells). The cells were cultured in HBSS media and three different concentrations i.e; 0.1mg/ml. 0.01mg/ml & 0.001mg/ml of lectins from different sources were added to the cells along with control. It was observed that the invertebrates showed mitogenecity and proliferation of 83.63% at 0.1mg/ml of lectin from Whole earthworm body having affinity with galactose that is with more concentration of lectin more proliferation was observed while vertebrates showed 85.7% in 0.001 mg/ml with lectin from MC that is with more concentration of lectin less proliferation was observed hence on vertebrates it has inhibitory effect (Imanishi *et al* 1981).

Table 1: protein estimation

Std Protein (BSA)	Protein concentrationObtained @ 540 nm
1.0 ml	1.00 mg/ml
0.75 ml	0.75 mg/ml
0.5 ml	0.50 mg/ml
0.25 ml	0.25 mg/ml

Lectin sample	Protein concentration
CC glu	1.78 mg/ml
CC gal	1.29 mg/ml
MC glu	1.52 mg/ml
MC gal	1.36 mg/ml
WE glu	1.23 mg/ml
WE gal	1.17 mg/ml
VC glu	0.87 mg/ml
VC gal	0.84 mg/ml

Table 2: Sugar binding specificity
(A): Glucose binding ability of tested lectins (mg/ml)

Lectin	sugars	mg/ml protein bound to sugar
CC	glucose	0.952
MC	glucose	0.841
WE	glucose	0.730
VC	glucose	0.954

(B): Galactose binding ability of tested lectins (mg/ml)

Lectin	sugars	mg/ml protein bound to sugar
CC	galactose	0.801
MC	galactose	0.950
WE	galactose	0.860
VC	galactose	0.855

Table 3: SDS-PAGE

Sample	Mol. Wt. (KDa)
Coelomocytes	26KDa
Muscle cells	26KDa
Whole earthworm	26KDa
vermicompost	26KDa

Table 4: Hemagglutination

Blood group	Agglutination
A $^{+ve}$	No
B $^{+ve}$	No
AB $^{+ve}$	No
O $^{+ve}$	No

Table 5: Antimicrobial activity
A) Bacteria

Micro Organism (Bacteria)	Volume of samples in µl from mg/ml concentration	Zone of inhibition (in cms) for 8 different protein samples							
		CC gal	CC glu	MC gal	MC glu	VC gal	VC glu	WE gal	WE glu
Staphylococcus aureus	50	1.4	1.2	-	1.2	1.8	-	-	-
	100	1.9	-	-	1.5	-	-	-	-
	150	2.6	1.6	-	2.4	2.4	-	-	-
	200	2.6	2.0	-	2.0	2.6	-	-	-
Bacillus subtilis	50	-	1.8	1.5	-	1.4	-	-	-
	100	-	2.0	2.3	-	1.4	-	-	-
	150	-	-	2.4	-	1.5	1.5	-	-
	200	-	-	2.4	-	-	2.0	-	-

B) Fungus

Micro Organism (Fungus)	Volume of samples in µl from mg/ml concentration	Zone of inhibition (in cms) for 8 different protein samples							
		CC gal	CC glu	MC gal	MC glu	VC gal	VC glu	WE gal	WE glu
AspergillusNiger	50	0.3	0.5	-	0.6	0.2	-	-	-
	100	0.5	0.2	0.7	-	0.3	0.5	-	-
	150	0.7	-	0.9	0.5	0.6	0.3	-	-
	200	0.9	-	-	-	1.0	-	-	-
Penicillium notatum	50	1.0	0.9	0.3	1.0	0.2	1.1	0.2	0.5
	100	1.2	1.2	0.5	0.6	0.4	0.9	0.6	0.7
	150	1.3	0.8	0.7	0.6	0.5	1.3	0.8	0.8
	200	1.5	1.2	1.1	0.5	0.6	1.5	1.0	0.7

Table 6: Mitogenic activity
A) For earthworm muscle cells (invertebrates):

S. No.	sample	Control % viability	0.1mg/ml	0.01 mg/ml	0.001mg/ml
1.	CC glu	92.30%	83.30%	77.77%	60.00%
2.	CC gal	88.88%	76.66%	68.82%	55.50%
3.	MC glu	58.14%	66.66%	62.50%	60.00%
4.	MC gal	66.60%	66.60%	65.21%	57.14%
5.	WE glu	60.00%	64.70%	62.5%	60.55%
6.	WE gal	68.29%	83.63%	72.33%	69.20%
7.	VC glu	61.70%	68.66%	62.92%	60.58%
8.	VC gal	60.60%	67.85%	64.70%	60.68%

B) For earthworm muscle cells (vertebrates)

S. No.	sample	Control % viability	0.1mg/ml	0.01 mg/ml	0.001mg/ml
1.	CC glu	67.00%	54.40%	58.00%	63.50%
2.	CC gal	80.00%	68.18%	75.00%	78.12%
3.	MC glu	66.66%	60.00%	63.00%	65.20%
4.	MC gal	94.60%	77.27%	81.25%	85.71%
5.	WE glu	75.00%	52.63%	60.00%	70.00%
6.	WE gal	81.25%	73.07%	77.77%	79.16%
7.	VC glu	63.63%	45.45%	40.00%	66.66%
8.	VC gal	79.50%	74.00%	75.00%	78.50%

Conclusion

The Research work has been carried out with the aim of studying and characterizing some of the important properties exhibited by lectins and their specificity depending on the specific source from which it is isolated. The lectin is found in the coelomocytes (coelomic fluid), muscle cells, whole body (gut) and in the vermicompost of earthworm. Lectin showed affinity to closely related sugars like glucose and galactose and showed no agglutination with human blood type. The molecular weight of all lectins was found to be 26 KDa

Antimicrobial activity showed that lectins have inhibitory effect on *Bacillus subtilis Staphylococcus aureus*, *Aspergillus niger* and *Penicillium notatum* while there was no inhibition in case of *E. coli* and *Fusarium oxysporum.* Lectins also showed mitogenic activity on invertebrate earthworm muscle cells while they showed inhibitory effect on vertebrate chicken liver cells.

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References

Cooper, E. L., Cossarizza, A., Suzuki, M. M., Salvioli, S., Capri, M., Quaglino, D. and Fransceschi, C. 1995. Autogeneic but not allogeneic earthworm effector coelomocytes kill the mammalian tumor target K562. *Cellular Immunology,* **166:** 113-122.

Dodd, R. B. and Drickamer, K. 2001. Lectin-like proteins in model organisms: implications for evolution of carbohydrate-binding activity. *Glycobiology,* **11:** 71-79.

Ferdinand, Rose. 1833. Über die Verbindungen des Eiweiss mit Metalloxyden. *Mathematical-natural sciences*, **24:** 335-337.

Hrzenjak, M., Kobrehel, Đ., Levanat, S., Jurin, M. T., Hrzenjak. 1993. Mitogenicity of earthworm (*Eisenia foetida*) insulin-like proteins. Comp. Biochem. *Physiol,* **104:** 723-729.

Imanishi, K., Ishiguro, T., Saito, H. and Suzuki, I. 1981. Pharmacological studies on a plant lectin, Aloctin A. I. Growth inhibition of mouse methylcholanthrene-induced fibrosarcoma (Meth A) in ascites form by Aloctin A. J. cellular and molecular life sciences, Volume 37: *Number*, **11:**1186-1187.

Kilpatrick, D. C. 2002. Animal lectins: an historical introduction and over-view, *Biochim Biophys Acta*, **1572:** 187–197.

Karel, L. and Franklin, R. C. 1946. J. Cell. and Comp. *Physiol*. **28:** 349

Liao, W. R., Lin, J. Y., Shieh, W. Y., Jeng, W. L. and Huang, R. 2003. Antibiotic activity of lectins from marine algae against marine vibrios. J Ind Microbiol Biotechnol., **30:** 433–439.

Licata, A. and Mauceri, L. 2002. Lectin histochemistry of epidermal glandular cells in earthworm *Lumbricus terrestris*. *Eur J Histochem*, **46:**173-178.

Miller, G.L., 1959. Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Anal*. *Chem,* **31:**426

