STUDIES ON THE AGGLUTININS FROM THE HEMOLYMPH AND MUSCLE TISSUE OF SEA CRAB *CALAPPA RUBROGULATTA*

Oladimeji, S. O.
Department Of Biochemistry Lagos State University, Ojo
P.m.b. 1087 Apapa, Lagos, Nigeria

ABSTRSACT
Agglutinin were isolated from the hemolymph and muscle tissue of sea crab (Calappa rubrogulatta) and purified over a SE-sephadex gel (cation exchanger). This was followed by gel filtration using sephadex G200 gel. The agglutinins from the two tissues were found to be similar to those previously studied in other invertebrates. The agglutinins reacted with purified peanut Arachis hypogea forming a preciptin line in an immunodiffusion assay. The hemolymph and muscle tissue extract have molecular weight of 45.7 and 64 kilodattons (kda) sub units respectively on a dissociating gel electropheresis.

Key Words: Agglutinins, sea crab, hemolymph, muscle tissue

INTRODUCTION

Invertebrates have been demonstrated to have natural agglutinins with specificity towards certain vertebrate erythrocytes (Finstad et al 1992). The presence of agglutinins in fresh water crabs Limulus Polyphemus has been studied (Boyd and Shapleigh, 1954). The agglutinins had earlier been described as a non-immune sugar binding protein, which agglutinate cells and /or precipitate glycoconjugate (Sharon and Lis, 1975; Barondes, 1998).

Agglutinins of invertebrate source have been isolated and purified from the hemolymph and other tissues extract and are proposed to function in immune or sugar transport system invivo (Den and Malinzak, 1997, Yeaton 1981) The high affinity of the agglutinins for specific sugar residues was compared in a similar degree to which antibodies are specific for their antigen depending on level of its purity (Alaba et al 1988) Agglutinins have been shown to posses sub units that are non covalently linked and required Ca^{+2} ions for the expression of biological activity, (Marchalonis and Edelman, 1998). The objective of this study was to purify and characterize the hemolymph and muscle tissues of Callapa rubrogulatta This was with a view to establish presence of the agglutinins in a sea crab specie and to demonstrate similarities or differences in the properties of the agglutinin within tissues.

MATERIALS AND METHODS

Source Of Materials:

Sea crab, *Calappa rubrogulatta* were harvested from the Lagos Lagoon by a thrawler fishing company were they were purchased. Red blood cells used were collected from Rabbit, Sheep and human ABO blood groups donours by vein puncture. (Fritz and Malinzak 1977).

Extraction:

Hemolymph from *C. rubrogulatta* were extracted from the pericardium as earlier reported (Fritz and Malinzak 1977). The hemolymph was allowed to clot and later centrifuged and stored at -20°C until when needed. The agglutinins from the muscle tissues were extracted from the claws of the crabs as described by Sharon and his, (1975).

Erythrocytes Cell Suspension:
Fresh sterile sheep, rabbit and human ABO blood groups were washed three times and suspended as earlier reported Hammarstom and Kabat (1999)

Microtiter Assay Method:
Agglutinins activity was assayed by the microtiter method as previous described. (Hammarstrom and Kabat (1999)

Purification
A stepwise 0-80% $(NH)_2 SO_4$ (Ammonium Sulphate) saturation was carried out to precipitate the agglutinins from the two crude extract. The precipitates were later extensively dialysed against distilled water in a dialysed tubing (Marchalonis and Edelman 1998). The different $(NH)_2 SO_4$ saturation solution was centrifuged at 30,000g for 2 hours, at 4° C.

A two step chromatography were carried out on the active agglutinins dialysate of the hemolymph and muscle tissue extracts using se-sephadex gel and sephadex G200 procedure as carried out by earlier workers (Hamarstrom and Kabat, 1999; Barondes 1978).

Electrophoretic Analysis
The method of Salton (1997) was used in determining the purity level of the agglutinins extracts.

Total Protein And Carbohydrate Content
Protein content was determined by Folin C reagent (Lowry et al 1957) Carbohydrate was determined with phenol-sulphuric reagent (Aminoff, 1961 and Dubios et al 1956).

Sugar Inhibition Of Agglutination
Two fold serial dilution with sugar inhibition in microtitre agglutination assay was as described by (Goldstein and Hayer 1978, Beeley 1985).

Immunodiffusion Assay
A 1% Agarose double diffusion assay was as described earlier (Goldstein and So, 1995).

RESULTS
The purification table (Table 1) confirmed that the extracts were agglutinins and were made up of protein and carbohydrate. The agglutination reaction of the purified agglutinins with rabbit and human B blood group erythrocytes, gave a microtiter value of 64, as the reciprocal of highest dilution that gave visible agglutinins in a two fold serial dilution assay. The specificity of the agglutination to the erythrocytes cells is similar to those obtained from the agglutination activity of the purified Limilus Polyphamus and Asterias forbesi agglutinins which showed agglutination with horse erythrocytes, (Finstad et al, 1974).

The agglutinating inhibition assay showed microtiter value of 2 for glycosamine and Xylose as compared to the mictotiter value of 64 for other monosaccharide without inhibition. The presence of these sugars as part of the carbohydrates moiety of the agglutinin was further demonstrated, when identified on the paper chromatography in line with the work of (Goldstein and Hayer, 1978).

TABLE: PURIFICATION TABLE

	Carbohydrate mg ml	Protein mg/ml	Hemaglutinating titer value	Specific activity-titer mg/ml	Purification Fold	Yield
Samples:						
Crude supernatant					1	
A) Hemolymolymph					1	100
B) Muscle extract						
0-50% Saturation (NH_4) SO4 precipitate						100
a) Hemolymph	17.40	0.920	8	8.7		
b) Muscle extract	9.60	0.420	8	19.06		
70% saturation $(NH_4)2SO4$ precipitate	14.00	0.310	a	a		
a) Hemolymph	8.80	0.089	a	a		
b) Muscle extract						
80% Saturation $(NH_4)2SO4$ Precipitate	7.80	0.340	a	a		
a) Hemolymph	3.00	0.130	a	a	14.16	
b) Muscle extract					16.80	
Active fraction on sephadex G200	0.48	0.138	16	123.07		
a) Hemolymph	0.47	0.056	16	320	92.05	
b) Muscle extract					112.04	
Active fraction on SE-sephadex	0.16	0.088	64	800		14%
a) Hemolymph	0.03	0.033	64	2133.33	147.29	11%
b) Muscle extract					168.06	
	0.07	0.056	64	1280		8%
	0.06	0.022	64	3200		7%
						5%
						4%

Note (1) Estimate done in duplicates.

a = not detected

The elution pattern recorded for the two extracts over SE-sephadex G200 as shown in figures 1 and 2 showed two peaks, 1 and 2 corresponding to two major proteins eluted. The obtained elution curve on figures 3 and 4 for the extract over sephadex G200 showed a major agglutinating peak 3.

A major single band was observed on the polyacrylamide gel eletrophoresis of the purified agglutinins indicating electrophoretic mobility and level of purity. The SDS PAGE result in figure 5 for the agglutinins showed, two distinct bands which implied that the agglutinins were made up of two sub unit with estimated molecular weights of 45.7 kda and 64.4 kda.

The results obtained on the agarose gel double immunodiffusion assay with the agglutinins of the two extract showed a precipitin line with purified glycoprotein of groundnut (Arachis hypogea) as shown in figure 6. The observed precipitinin line agrees with the location of the agglutinins activity for both purified extracts.

DISCUSSION AND CONCLUSION

These studies revealed a striking consistency in agglutination reactions of Calappa rubrogullata agglutinins with those of other invertebrates . It was equally demonstrated that the agglutinins being studied was a glycoprotein with some degree of specificity in it's agglutinating activity. This specificity of reaction was further emphasized with a cross reactivity of the agglutinins with a cross reactivity of the agglutinins with purified peanut hypogea glucoprotein. The estimated sub unit molecular weight of the active agglutinins were found to be same for the hemolymph and muscle tissue extracts.

From these analysis it was concluded that sea crab have agglutinins with relatively equal distribution in hemolymph and muscle tissues. The biological specificity of these agglutinins are seemingly similar in both tissues.

ACKNOWLEDGEMENT.

The author is grateful to Mrs. Ester Abiola for the purchase of some vital biochemicals used. The gesture of all blood donors and the Animal house, college of medicine Idi-araba, Lagos is also highly appreciated.

REFERENCES

1. Alaba, A., Coker A.O. and Okotore, R.O. (1988) Interaction of Lectins with the surface coasts of campylobacter species in Nigeria. Clinical Biochem. 6: 565 – 70.
2. Aminoff, D. (1961): Paper Chromatographic Analysis Biochem. J. 81, 384 – 388
3. Barondes S.H. (1978) Developmentally regulated slime mould lectins and specific cell cohesion. In: The molecular basis of cell to cell interaction. Lrnes R.a. and Bergsma D. eds Alan R. Liss, New York. 24. 491 – 496.
4. Barondes S.H. (1978): Lectins – Their multiple endogenous cellular functions. Ann. Rev. Biochem. 50, 207 – 231.
5. Beeley, J.G. (1985) Glycoprotein and Proteoglycan technique in "laboratory Techniques in biochemistry and molecular Biology "R.H. Burdon and P.H. Van Knippeuderg{ed} 462p. Elsevier New york.
6. Boyd, W.C. and Shapleigh, E. (1954): Specific precipitating activity of plant agglutinins {lectins}, Science, 119, 419 – 421.
7. Den,H. and Malinzak, D,A (1977); Isolation and properties of beta D-galactose specific lectin from chick embryo thigh muscle. J. Biol. Chem. 252, 5444 – 5448
8. Dubios M: Giles, K.A.: Hamilton, J.K., Reers, P.A. and Smith F. (1956): Colorimetric methgod for the determination of sugars and related substances. Anal, Chem. 28, 350 – 356.
9. Finstad, C.L. Litman, G.W., finstad J. and Good R.A. (1992). Agglutinins from invertebrates. J. Immunol vol. 108. 1704 - 1711
10. Finstad, C. L., Good, R.A. and Litman, G.w. (1974): the erythrocytes agglutinin from Limilus polyphamus hemolymph: Molecular structure and biological function. Ann. N.Y. Acad. Sci. 235, 170 – 182.
11. Fritz, H. and Malinzak, D.A. (1977): Isolation of proteins : Method of Enzymatic Analysis, vol. 2 Bergmeyer, H.U. {eds} P.1064 – 1070.
12. Goldstein I.J. and Hayer, G.E. (1978): The lectins carbohydrate Binding proteins. Adv. Carbhy. Chem. Biochem and biochem. 35, 145 -149
13. Goldstein I.J. and So, I.L. (1995). Protein – Carbohydrate interaction Agar-gel difussion studies on the interaction of concavalin A, a lectin isolated from Jack – bean with polysaccharides. Biochem. Biophys. Acta 111, 407 – 414.
14. Hammarstrom, S. and Kabat, E.A., (1919): the characterization of purified erytrocytes agglutinins. Biochemistry, 8:2692 – 2699.

15. Lowry, O.H.; Rosebrough, N.J. Farry, A.L. and Randall, R.J. (1951). Protein measurement with the folin phenol reagent J. Biol. Chem. 193, 256–275

16. Marchalonis J.J. and Edelman, G.M. (1998). Purification of a hemagglutinin from Limulus polyphemus. *J. Mol. Biol.*, 32, 453 – 465.

17. Salton, M.R.J. (1997). Electrophoretic mobility studies on glycoprotein using polycylamide Gel Electrophoresis N.Y. Acad. Sci 24, 764 – 781.

18. Sharon, N. and Lis, A (1975). Use of letins for the study of membranes. In: Methods in membrane *biology, plasma membranes*, Korn Ed., Plenum, New York 3, 147 – 200.

19. Yeaton, R.W. (1981). Sugar specific lectins in the hemolymph of solitary ascidian. Dev. Comp. Immunol. 5, 535 – 545.