OBAFEMI AWOLOWO UNIVERSITY

PURIFICATION, CHARACTERIZATION AND ANTIMICROBIAL ACTIVITIES OF THE LECTIN IN THE SKIN MUCUS OF AFRICAN CATFISH (*Clarias gariepinus*, Burchell, 1822).

BY

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CERTIFICATION

This research project was supervised by us and approved in accordance with the requirement for the award of Doctor of Philosophy (Ph.D) Degree in Biochemistry, Obafemi Awolowo University, Ile-Ife, Nigeria

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DEDICATION

The work is dedicated to my beloved late father, Solomon Olaposi Odekanyin, who laid the foundation of my education, my living loving mother, Comfort Oluwaremilekun Odekanyin, who built steadily on the foundation laid by her husband, and finally to **GOD:** My creators and **The Lord Jesus Christ**: My savoiur.

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ABBREVIATION

A_{280}	Absorbance at 280
BSA	Bovine Serum Albumin
Con A	Concanavalin A
CRD	Carbohydrate Recognition Domain
CTL	C-type Lectin
DEPC	Diethyl Pyrocarbonate
DTNB	5, 5' – Dithiobis-(2-nitro benzoic acid)
EDTA	Ethylenediamine tetraacetic acid
GF-1	Gel Filtration Peak-1
GF-2	Gel Filtration Peak-2
NBS	N-Bromosuccinimide
PAGE	Polyacrylamide Gel Electrophoresis
PBS	Phosphate Buffered Saline
PHA	Phytohaemagglutinin
PMSF	Phenylmethyl Sulphonyl Fluoride
RBC	Red Blood Cell
SDS	Sodium Dodecyl Sulphate
TEMED	N',N',N',N' Tetramethylethylene Diamine
Tris-HCl	2-Amino-2-(hydoxymethyl) propane-1,3-diol-hydrochloric acid
WGA	Wheat Germ Agglutinin

ABSTRACT

The study purified a galactose-specific lectin from the skin mucus of African catfish (*Clarias gariepinus*) and determined the physicochemical properties and antimicrobial activities of the lectin. This was with a view to examining the involvement of the protein in the host defense mechanism and the adaptability of the fish to diverse environmental conditions.

African catfish (*Clarias gariepinus*) was obtained from the Osin Fish Farm, Yakoyo via Ile-Ife. The skin mucus was scraped and homogenized in 10 mM phosphate buffer, pH 7.2 containing 50 mM NaCl to obtain the crude extract. The blood and sugar specificities of the lectin in the extract were determined. The lectin was purified to homogeneity by a two-step purification procedure - gel filtration on Sephadex G-150 and affinity chromatography on Lactose-Sepharose 4B column. The effects of pH, temperature, Ethylenediamine tetraacetic acid (EDTA) and divalent cations such as Ba^{2+}, Ca^{2+} , Mn^{2+} , Mg^{2+}, Fe^{2+} and Sn^{2+} on the haemagglutinating activity of the lectin was investigated using standard methods. The molecular weight of the native protein and the subunit was estimated by gel-filtration on Sephadex G-100 and Sodium Dodecyl Sulphate – Polyacrylamide gel electrophoresis (SDS-PAGE) respectively. The amino acid composition of the protein was determined using standard methods. The presence of covalently-bound carbohydrate in the protein was investigated with Periodic Acid-Schiff's reagent (PAS Staining). Ouchterlony double diffusion technique in agar gel was carried out to examine the interaction of the lectin with carbohydrates. The antimicrobial activities of the skin mucus lectin of catfish were investigated by identifying and characterizing different isolates of pathogens in the fish environment. The ability of the lectin to agglutinate and suppress bacteria growth was tested and the inhibitory effect of the lectin on the mycelia growth of the fungi isolates was investigated.

Phosphate buffered saline extract of the skin mucus of African catfish specifically agglutinated erythrocytes of rabbit and human blood group B, but did not agglutinate bat, rat, hen and human blood A and O erythrocytes. The haemagglutinating activity of the lectin was completely inhibited by lactose and slightly inhibited by galactose and melibiose and was calcium-independent. The purified lectin has a native and subunit molecular weight of 63, 000 daltons and 20, 000 daltons respectively suggesting a trimeric structure for the protein. The protein contained 126 amino acids residues per subunit. This was characterized by large amount of polar amino acids that constituted about 60 % of the total amino acids. The lectin showed maximum activity over the pH range 6 – 9 and was heat stable up to 50 ^{o}C. EDTA had no inhibitory effect on its haemagglutinating activity. PAS staining showed that the lectin was not a glycoprotein. Chemical modifications of serine and arginine residues of the protein did not affect its haemagglutination activity while modifications of cysteine, tryptophan and histidine residues led to total loss of its activity. The microbial load of the fish pond water was high, with highest total bacterial count of 7.1 x 10^{8} cfu/ml and fungal count of 6.7 x 10^{3} cfu/ml. Twelve bacterial species and 14 fungi species were isolated and identified from the pond water. The lectin specifically agglutinated different Gram-negative bacteria such as *Aeromonas hydrophila, Alcaligenes faecalis, Klebsiella edwardsii* and *Vibrio metschnikovii,* but had no effect on Gram-positive bacteria. It inhibited strongly the growth of *A. hydrophila, A. faecalis, Bacillus cereus, B. polymyxa, K. pneumoniae, K. edwardsii, Pseudomonas aeruginosa and V. metschnikovii.* In the same manner, the lectin inhibited the mycelia growth of yeast, *Kluyveromyces marxianus* and a phytopathogenic fungus, *Fusarium oxysporum.*

The study concluded that African catfish skin mucus lectin exhibited similar physicochemical properties with lectins from other fish skin mucus. The lectin was involved in the innate host defense mechanism of the fish.

CHAPTER ONE

INTRODUCTION

1.1 Background to the Study

The occurrence in nature of erythrocyte-agglutinating proteins has been known since the turn of the 19th century. By the 1960's, it became apparent that such proteins also agglutinate other types of cells and that many of them are sugar-specific. These cell-agglutinating and sugar-specific proteins have been named lectin (Sharon and Lis, 2004)

The term lectin was derived from the Latin word "legere" which means to choose, pick out or to select and was first proposed by Boyd and Shapleigh (1954) to designate the ability of plant agglutinins to distinguish between erythrocytes of different blood types. Several definitions of lectins have been given during past years, but the currently accepted definition of lectin is related to function rather than structural criteria (Sharon and Lis, 2004). Thus, lectin is defined as a carbohydrate-binding protein or (glyco)protein of non-immune origin, containing at least one non-catalytic domain, which agglutinates cells (erythrocytes), precipitate glycoconjugates and polysaccharides and is capable of specific recognition of and reversible binding to carbohydrate moieties of complex glycoconjugates without altering the covalent structure of any of the recognized glycosyl ligands (Sharon and Lis, 2004).

Generally, lectins are a heterogeneous class of proteins that bind specifically and reversibly to carbohydrates (Sharon and Lis, 1989a). They can recognize mono-, oligo-, or poly-saccharides, as well as glycoconjugates and thus recognize glycoproteins and glycolipids on the surfaces of cells (Sharon and Lis, 1995). There are multivalent lectins that carry two or more carbohydrate-binding domains; these lectins are, therefore able to agglutinate cells or to

precipitate glycoconjugates (Reeke and Becker, 1988; Chrispeels and Raikhel, 1991). Because of their agglutination activities, lectins have been originally described as cell agglutinins. To be considered as a lectin, a given protein or glycoprotein must fulfill three requirements: it must bind to carbohydrate; it must be distinct from immunoglobulins and it must not biochemically modify the carbohydrate which it binds (Goldstein *et al.,* 1980; Sharon and Lis, 1989a; Gabius, 1997; Rudiger and Gabius, 2001; Duranti, 2006).

Another definition given to lectins is that they are sugar-binding proteins that are neither antibodies nor enzymes (Sharon and Lis, 1972). They are widely distributed in nature and can be found in almost all living organisms including bacteria, fungi, plants, invertebrates and vertebrates and may be either soluble or membrane-bound (Lis and Sharon, 1986). These proteins can usually be detected by agglutination activity against a variety of animal cells, such as erythrocytes, by virtue of their ability to bind to cell surface glycoconjugates. This is based on the nature of lectins, having one or more carbohydrate recognition domain (CRD) (Doyle and Keller, 1984).

Plants are the richest source of lectins, with several hundred well studied to date. The lectins have been found in a large number of plant species encompassed by many of the major taxonomical grouping of the plant kingdom (Goldstein and Hayes, 1978). Plant lectins are a rather heterogeneous group of proteins which have little in common apart from their ability to recognize and bind specific carbohydrate. However, a close examination of the available data reveals that the majority of all currently known lectins can be classified into four major groups of structurally and evolutionally related proteins. These four groups are the legume lectins (Sharon and Lis, 1990); the chitin-binding lectin composed of hevein domains (Beintema, 1994; Trindade *et al.,* 2006), the so called type-2 ribosome-inactivating proteins (RIP) (Barbieri *et al.,*

1993) and the monocot mannose-binding lectins (MBL) (Ding *et al.,* 2008; Barre *et al.,* 2001). Whereas the first group is strictly confined to the legume family, the latter three groups comprise lectins from species belonging to several plant families.

Since the discovery of mammalian liver lectin in 1974, a large number of researchers have intensively investigated the animal lectins. Based on the structure of the CRD, animal lectins are classified into several families (Gabius, 1997; Kilpatrick, 2002), such as S-type (galectin) specific to beta-galactosides (Barondes *et al.,* 1994; Kasai and Hirabayashi, 1996), C-type that shows Ca^{2+}-dependent activity (Day, 1994), P-type (Kornfeld, 1992), I-type (Powell and Varki, 1995), L-Rhamnose-binding lectins (Tateno *et al.,* 2001), heparin-binding proteins (Margalit *et al.,* 1993, and pentraxin (Steel and Whitehead, 1994).

Some animal lectins are believed to act as defensive molecules. For example, the mannose receptor of mammalian macrophages and hepatic endothelial cells mediates phagocytosis of pathogenic agents (Stahl, 1992). Selectins found on platelets and endothelial cells mediate the initial adhesion of moving leucocytes to the stationary endothelium near inflamed tissues (Springer, 1994). Galectins modulate the activity of the macrophage membrane receptor for complements C3b and iC3b (Avni *et al.,* 1998). Furthermore, it should be noted that lectins especially mannose-binding lectin (MBL), which is one of the collectins characterized by their collagen-like domain, play an important role as initiator in complement cascade. MBL shares sequence homology to complement factor C1q, and activates complement system via the so-called lectin pathway (Ikeda *et al.,* 1987; Ohta *et al.,* 1990). In the case of fish species, MBL-homologue was cloned from the spleen of three Cyprinidae species (the zebrafish, *Daino rerio*; the carp, *Cyprinus carpio* and goldfish, *Carassius auratus*) (Vitved *et al.,* 2000), although their functions have not yet been clarified. On the other hand, opsonic serum lectins are found in Atlantic salmon, *Salmo salar* (Ottinger *et al.,* 1999; Ewart *et al.,* 2001) and blue gourami, *Trichogaster trichopterus* (Fock *et al.,* 2001). In addition to these lectins, fish egg lectins in steelhead trout, ***Oncorhynchus mykiss*** (Tateno *et al.,* 2002), chum salmon, *Oncorhynchus keta* (Shiina *et al.,* 2002) and common carp, *Cyprinus carpio* (Galliano *et al.,* 2003) also have been reported to bind to some bacterial species. Spleen, serum and egg lectins act internally, whereas other lectins are found