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Efficacy and Suitability of Lectin from *Mucuna sloanei* Seeds Extracts as a Cell Receptor Signal Inducer

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Abstract: The efficacy and suitability of lectin from *mucuna sloanei* seeds extracts as a cell receptor signal inducer was tested by its agglutinating ability in human and various blood cells such as goat, cow and chicken. The results showed that the isolated lectin from *mucuna sloanei* seeds extracts agglutinated human ABO, goat and chicken red blood cells at minimal concentration, 11.5 mg mL^{-1}, but did not agglutinate those of cow. It was also found that the physicochemical properties of the lectin did not affect agglutination by variation of the pH of the medium or affected by temperature. This suggests that the *mucuna sloanei* plant could be considered a good molecular marker for the study of blood group substances.

Key words: Lectin, mucuna sloanei, agglutination, erythrocytes

INTRODUCTION

Lectins are glycoproteins found in a wide range of plants and animals and microorganisms that interact with specific carbohydrate residues. The distribution of lectins within seed differ among the various plant families. Many lectins have been grouped into distinct families of homogenous protein with common structural properties and of these the members of the leguminosae have been most extensively studied (Eneobong and Carnoval, 1992; Shangary *et al*., 1994). The appearance of lectins in the seeds of most legumes occurs during the last stages of maturation of the seeds prior to their rehydration and most are localized in the cotyledons and have specificity for x-mannose and high mannose structures (Eyo *et al*., 1985).

Mucuna sloanei (Efik-Ibabat, Yakurr-Mpokpo, Igbo-Ukpo) is a leguminous plant which belongs to the leguminosae. It has several species and the leaves are usually alternatively trifoliate and flowers have superior ovaries containing mostly 3-4 seeds. The fruits are pods and split along the dorsal sutures when mature. The seed coat is black when mature but white to cream while young. The seeds are usually without endosperm and appear whitish (Eyo *et al*., 1985).

Mucuna sloanei seeds contain high protein and crude fats contents, thus potentiating the seeds as good source of meal as well as thickener, flavour agent and preservative, with high water binding capacity arising from the formation of hydrogen bonds between water and polar residues of the protein molecule. This explains why the seeds are used as soup thickener. The local community (Efik in Cross River State) claims that it lowers libido in men and has sedative properties. The mineral content include high potassium, calcium but low sodium and vitamin C, thus, it can be used as low sodium diet for obese patients. The total sugar content consists of glucose, fructose, mannose and arabinose. It also has alkaloid properties with curare-like action and can deter animals from eating the leaves and seeds (Eyo *et al*., 1985).

Lectins are useful reagents for the detection of the type and number of glycoproteins present on cellular membranes and therefore used to test the role of carbohydrate-containing substances in many membrane mediated processes. Lectins recognize and bind to specific sugars, including those complex carbohydrate-containing materials such as polysaccharides and glycoproteins. Lectins stimulate the division of the immune system known as lymphocytes and often synthetize antibodies, leading to mitogenesis. That could facilitate understanding of the relation between chromosome abnormality and human disease. Lectins also aid in the biochemical examination of processes involved in converting a resting cell into an actively growing one, hence, gene expression during the cell cycle (Zenteno *et al*., 1995). Thus, their mitogenic properties could indicate an ability to control cell division

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and germination in plants. This study, therefore, investigated the efficacy and suitability of this lectin as a cell receptor signal inducer by its agglutinating ability in human and various blood cells such as goat, cow and chicken.

MATERIALS AND METHODS

Preparation and extraction of sample: The seeds of *mucuna sloanei* were purchased at the Watt Market Calabar, Nigeria. The seeds were shelled and ground using an electric grinder. The powdered meal was defatted with petroleum ether using soxhlet extractor at room temperature. 2.50 g of the defatted meal was adjusted to 10% (w v^{-1}) with K-Pi (A) buffer and stirred overnight. The suspension was filtered through cheese-cloth and clarified by centrifugation at 2000xg for 20 min. The pH of the supernatant (crude extract) was lowered to 4.3 by dropwise addition of 2M NH_4OAc, under constant stirring. A precipitate was formed and the mixture was stirred for 15 min at room temperature and centrifuged at 4000xg for 30 min. The pH of the supernatant was brought back to 6.4 by addition of 2M NH_4 OAc, pH 9.5; extensive dialysis was performed, first against water and then with K-Pi (A) buffer.

Haemagglutinating activity: Haemagglutinating activity was determined with modifications of the method of Stojanvic. Hunan red blood cells (groups ABO) were obtained from healthy donors in Calabar-while the blood of goat cow and chicken were procured from the slaughter house in Bogobiri, Calabar, Nigeria. Two mg mL^{-1} sample of the lectin solution was diluted serialy 2-fold in K-Pi (A) buffer in microlitre wells (test tubes). A 4% suspension of erythrocytes (15 mL) was added. Haemagglutination titre was determined after one hour (1 h) incubation at room temperature. The agglutination was observed with the naked eyes. The above procedure was repeated for type B and O human red blood cells; and also for the erythrocytes of goat, cow and chicken. One unit of agglutination was defined as the lowest concentration of lectin giving visible agglutination under the experimental conditions used.

The physicochemical properties of the lectin were also determined with modifications of the method of Stojanovic. Two milligram per millilitre of the lectin in K-Pi (A) buffer was exposed to 45, 55, 65 and 75°C. Haemagglutination titres were assayed after various incubation times.

Table 1: Haemagglutinating activity of mucuna sloanei lectin

Blood sample	Agglutination
Human A	+
Human B	+
Human O	+
Goat	+
Cow	-
Chicken	+

+ = Agglutination, - = No agglutination

Table 2: Effect of pH variation on agglutination

pH	Agglutination
3	+
4	+
5	+
6	+
7	+
8	+
9	+
10	+

+ = Agglutination

Table 3: Effect of temperature variation on agglutination

Temperature (°C)	Agglutination
45°	+
55°	+
65°	+
75°	+

+ = Agglutination

RESULTS

Table 1-3 present the results of the haemog-glutinating activity, effect of pH variation on agglutination and effect of temperature variation on agglutination of the isolated lectin from *mucuna sloanei* respectively. The results showed that the lectin agglutinated human ABO erythrocytes as well as those of goat and chicken but not those of cow. The results also showed that agglutination of erythrocytes was not remarkably affected by variation of the pH of the medium or affected by temperature.

DISCUSSION

Haemagglutinating activity is shown in Table 1. The crude extract as well as the dialysate showed agglutination. The lectin agglutinated human red blood cells of either type A, B, O, though A and O showed more strongly agglutination. It also agglutinated the red blood cells of goat and chicken but not those of cow. In the dialysate, there was a slight increase in haemagglutinating activity as compared to the crude extract. This is probably due to the removal of inhibitors initially present in the crude extract. Agglutination of human red blood cells (ABO) signified the presence of recognizable sugar residues, perhaps N-acetyl-D-glucosamine or relative derivation of the erythrocyte membranes by the lectin. It also signified that it is a human red blood non-specific lectin.

Cell membrane is an assemblage of lipids and proteins. Proteins molecules lie on the surfaces of the lipid bilayer or are embedded in it. This membrane architecture forms the framework for cell agglutination, crosslinking of cells by lectins that bind to specific receptors, sugar units of oligosaccharide chains protruding from the cell surface. This clustering of the receptors indicates a change in the fluidity of the membrane, which allows for the pulling together of the receptor and their diffusion through the lipid bilayer of the membrane. The increased fluidity, characteristic of malignant cells, could account in parts, for their decreased stickness and ability to migrate through the body from their tissues of origin. This could suggest that agglutination of lectin depends on relatively distribution of sugar receptors on the surface of cells where receptors are dispersed in normal cells and clustered in cells that have undergone malignant transformation or proteolysis. Thus, lectins may provide a useful tool for the study of chemical architecture of normal or transformed cell surfaces, for isolation of glycoconjugates and for use in other areas of biomedical sciences.

The physicochemical properties of the lectin were also investigated (Table 2 and 3) and the results showed that agglutination of erythrocytes was not remarkedly affected by variations of the pH of the medium. The lectin retained its activity through the pH range (3-10) tested or by temperature (45, 55, 65 and 75°C).

CONCLUSION

In this study, the isolated lectin from *mucuna sloanei* seed extracts has been found to possess the mitogenic properties within a concentration range. This suggests that this lectin possesses some therapeutic potential and could be used for mitogenic stimulation of normal human peripheral blood lymphocytes. This may also be useful in the diagnosis and treatment of certain diseases.

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