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Research Article

PHYSICOCHEMICAL CHARACTERIZATION OF AN HEMAGGLUTINATING PROTEIN FROM THE FRUIT OF *COLA NITIDA*, KOLA NUT

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ABSTRACT

Objective: This study was done to partially purify and characterize the hemagglutinating protein from the fruit of *Cola nitida.*

Methods: The crude extract of the fruit of *C. nitida* was subjected to 80% ammonium sulfate precipitation. Hemagglutinating activity was done by making serial dilution of the extract and protein in a U-shaped microtiter plate and incubating with erythrocytes of human ABO system. Sugar specificities were done by incubating the hemagglutinating protein with 0.2 M of various sugars. The effect of temperature, pH, and chelating agent was done by incubating the protein at different temperatures, buffers of varying pH and dialyzing against ethylene diamine tetraacetic acid (EDTA), respectively.

Result: This protein had hemagglutinating activity toward human erythrocytes (ABO system) and was non-blood group specific. The activity was stable at pH range of 5-12 and was inhibited at pH 3 and 4. The lectin activity was heat stable up to 70°C and was not inhibited by chelating with EDTA.

Conclusion: A hemagglutinating protein was found in the fruits of *C. nitida,* which was slightly inhibited by lactose and sucrose.

Keywords: Lectin, Kola nut, pH, Temperature, Ethylene diamine tetraacetic acid, Thermostable, Hemagglutinating, Agglutination.

INTRODUCTION

Lectins are carbohydrate-binding proteins of non-immune origin, containing at least one non-catalytic domain [1,2], which agglutinates erythrocytes, precipitate glycoconjugates, and polysaccharides [3,4] and is capable of specific recognition of and reversible binding to carbohydrate moieties of complex glycoconjugates [5,6]. Because of their binding specificity, lectins have the ability to serve as recognition molecules within a cell, between cells or between organisms [7]. They are widely distributed in nature and can be found in almost all living organisms including plants, animals (vertebrates and invertebrates), and microorganisms.

Lectins have attracted great interest because of their effects on various biological systems such as cell agglutination [8], homing of leukocytes, induction of mitosis in lymphocytes, interferon, and cytotoxicity [9-11]. By virtue of their exquisite sugar specificities, lectins are useful tools in widespread applications for monitoring the expression of cell-surface carbohydrates as well as for the purification and characterization of glycoconjugates [8,12].

Ever since the discovery of lectins, scientists have been intrigued by their possible roles. The ubiquitous presence of lectins in nature, and their ability to discriminate between closely related saccharides in solution and on cell surfaces provides a major stimulus for the continuing search of their physiological functions. A strong argument that lectins have such functions is their conservation throughout evolution as homologous protein families. Another argument is that many of them are developmentally regulated, and their appearance often coincides with distinct physiological changes in the life of an organism [13].

Kola nut is a fruit from the kola tree, a genus (Cola) of trees that are native to the tropical rainforests of Africa. Kola nut belongs to the plant family Sterculiaceae, having about 125 species of trees native to the tropical rainforests of Africa. Of these, two species are particularly very common among the Yorubas of South Western Nigeria; these are *Cola nitida* and *Cola acuminata* [14]. They are made up of 2% caffeine, as well as kolanin and theobromine which are all stimulants. Other principal active constituents in kola nut include tannins, phenolics, kolatin, betaine, protein, and starch. The caffeine-containing fruit of the tree is used as a flavoring ingredient in beverages. It produces a strong state of elation and well-being, enhances alertness and physical energy, elevates mood, suppresses appetite and hunger and can also be used as an aphrodisiac. The caffeine in the nuts also acts by expanding the bronchial air passages; thus, kola nuts are often used to treat whooping cough and asthma [15]. The objectives of this study were to extract lectin from the fruit of *C. nitida* and determine some of its physicochemical properties.

EXPERIMENTAL PROCEDURES

Materials

C. nitida fruits were purchased from the local market in Ado-Ekiti, Nigeria and authenticated by the IFE HERBARIUM, Department of Botany, Obafemi Awolowo University, Ile-Ife, Nigeria. Human blood groups A, B, and O were obtained from healthy donors in Afe Babalola University, Ado-Ekiti. All chemicals and reagents used were of analytical grades.

Ammonium sulfate precipitation

Kola nut fruits were grinded with mortar and pestle. The powder (10 g) was weighed and suspended in 100 ml of phosphate buffered saline (PBS) pH 7.2. The mixture was stirred for 3 hrs and kept overnight at 4°C. This was followed by centrifugation at 3000 rpm for 20 minutes. The supernatant (crude extract) was collected and stored at −20°C. Protein concentration was determined by the method of Gornall *et al.* [16] using bovine serum albumin as standard. The proteins present in the crude extract of the fruit of *C. nitida* were precipitated with 80% ammonium sulfate.

Hemagglutinating activity

Agglutination of the red blood cells by the crude extract of *C. nitida* was estimated as described by Adeniran *et al*. [17]. Briefly, a serial two-fold dilution of the sample in PBS, pH 7.2 was mixed with 50 µL of a 4%

suspension of red blood cells in a U-shaped microtiter plate arranged in rows of wells. The plate was left undisturbed for 1 hr to allow agglutination of erythrocytes. Hemagglutination titer was expressed as the reciprocal of the highest dilution showing visible agglutination of erythrocytes. The blood group specificity of the extract was established using red blood cells from human blood groups (ABO system).

Sugar specificities of the protein were done similarly with the hemagglutination test. Serial two-fold dilutions of sample in PBS, pH 7.2 were incubated with 50 µL of each sugar (0.2 M). The mixture was allowed to stand for 1 hr at room temperature and incubated with 50 µL of a 4% human red blood cell suspension. The hemagglutinating titers obtained were compared with the blank which contained serial dilution of the protein without sugar. The sugars used in this study are listed in Table 1.

Effect of temperature on hemagglutinating activity

The effect of temperature on the agglutinating activity of the hemagglutinating protein from kola nut was determined by carrying out assay at different temperatures as described by Kuku *et al.* [18]. Briefly, the protein was incubated in a water bath for 30 minutes at various temperatures: 10, 20, 30, 40, 50, 60, 70, 80, 90, and 100°C and cooled to 20°C. Hemagglutination assay was carried out as previously described.

Effect of pH on hemagglutinating activity

The pH stability study was carried out as described by Adeniran *et al.* [17] by incubating the samples at different pH values using the following buffer: 0.2 M sodium acetate buffer, pH 3-6; 0.2 M tris-HCl buffer, pH 7-9; 0.2 M glycine-NaOH buffer, pH 10-12 and assaying for hemagglutinating activity as previously described. The control values were the agglutination titer of the lectin in PBS, pH 7.2.

Effect of ethylene diamine tetraacetic acid (EDTA) and divalent catons

The lectin was analyzed for metal binding site by chelating with EDTA and incubated in a water bath at 20°C for 30 minutes in the presence of 25 mM of chlorides of various metals such as KCl, NaCl, $CaCl_2$, $MgCl_2$, $MnCl_2$, $FeCl_2$, $FeCl_3$, and NH_4Cl, and followed by hemagglutination assay.

RESULTS

Hemagglutination activity

Phosphate buffered saline extracts of *C. nitida* agglutinated human red blood cells non-specifically as shown in Table 2. The lectin isolated may be classified into the category of agglutinins that agglutinate erythrocytes of all human blood groups alike usually referred to as non-specific agglutinins.

Sugar specificities

The result of sugar inhibition studies to define the sugar specificities of the phosphate buffered saline extract of *C. nitida* is as shown in Table 1. Fructose and mannose had no effect on hemagglutination activity, whereas glucose, galactose, sucrose, and lactose slightly inhibited the activity of the hemagglutinating protein.

Each experiment consisted of 100 µl of serially diluted lectin in a U-shaped microtiter well. B-type red blood cells were used for the agglutination.

Effect of temperature

The hemagglutinating activity of *C. nitida* protein was stable until 70°C. The activity declined at 80°C, and there was further reduction in hemagglutination activity at 100°C (Fig. 1).

Effect of pH

The lectin activity of *C. nitida* was found to be stable at pH range of 5-12. The activity was less between pH 3 and 5 as shown in Fig. 2.

Effect of EDTA and divalent cations

EDTA was found to have no chelating effect on the protein. Moreover, the addition of metal cations showed no effect on the lectin activity.

Table 1: Sugar inhibition of hemagglutination by *Cola nitida* crude extract

Simple sugar	Titer
Glucose	2^7
Galactose	2^7
Fructose	2^8
Sucrose	2^6
Lactose	2^5
Mannose	2^8

Table 2: Blood group specificity of phosphate buffered saline extract of white kola nut

Blood group	Hemagglutination titer
A	2^6
B	2^8
O	2^8

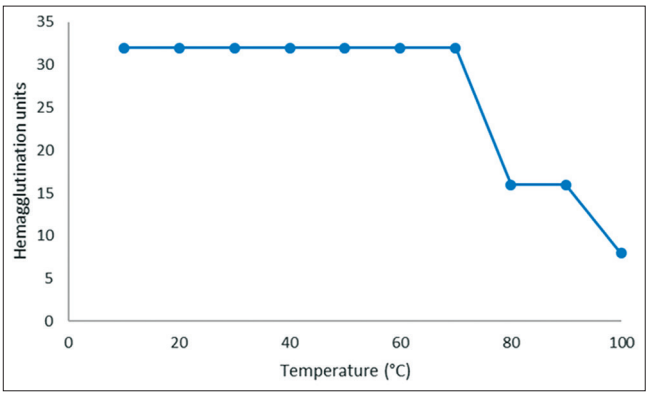

Fig. 1: Effect of temperature on hemagglutinating activity of *Cola nitida* lectin

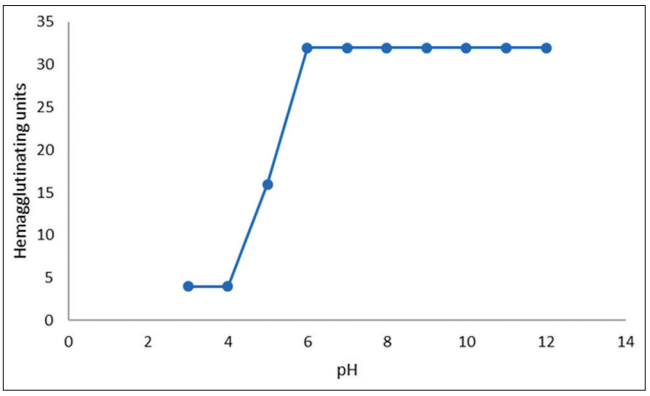

Fig. 2: Effect of pH on hemagglutinating activity of *Cola nitida* lectin

DISCUSSION

In the present study, the extract of the fruit of *C. nitida* showed considerable amounts of hemagglutinating protein and agglutinated human blood groups non-specifically. Hemagglutinating proteins are generally known to differ in reaction to thermal and pH characteristics. The activity of the hemagglutinating protein in this study was stable to 70°C, followed by decline in activity. However, there was still activity at 100°C indicating that the protein was thermostable. The study showed that *C. nitida* hemagglutinating protein was stable in the pH range 5-12. The result suggests that high acidic environmental conditions are not conducive for the hemagglutinating protein from the fruit of *C. nitida*.

Similar reports have been stated for many lectins in literature. pH dependence is a consequence of protein composition and is observed in virtually all enzyme reactions. Many ionizable groups at the surface of the protein molecule and the active center are capable of reacting with hydrogen or hydroxyl ions. Any change in pH is, therefore, associated with a change in the ionization state of the molecule, which, in turn, determines the binding forces between enzyme and substrate [19]. In this case, the increase in hydrogen ions caused a change in the ionization state of the lectin thereby affecting the binding forces between the lectin and erythrocyte membrane that eventually led to loss in activity. It may also be due to structural changes in the lectin at high acidic pH. Extensive dialysis of the lectin with EDTA did not bring about any change in hemagglutinating activity as well as incubation with metal cations, suggesting that either the lectin activity was not dependent on the metal cations or the metal ions are too strongly held in the lectin structure and could not be removed by dialysis with EDTA [17,20].

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