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Can γ-radiation modulate hemagglutinating and anticoagulant activities of PpyLL, a lectin from *Phthirusa pyrifolia*?

Romero M.P.B. Costa[a,*], Wendell Wagner Campos Albuquerque[a], Mariana C.C. Silva[c], Raiana Apolinário de Paula[b], Mychely Sheila Melo[b], Maria L.V. Oliva[c], Ana Lúcia Figueiredo Porto[a]

[a] *Laboratory of Technology in Bioactives Products, Rural Federal University of Pernambuco – UFRPE, Dom Manoel de Medeiros street, s/n, Dois Irmãos, CEP: 52171-900, Recife, PE, Brazil*
[b] *Department of Biochemistry, Center of Biologicals Sciences, Federal University of Pernambuco (UFPE), Av. Prof. Moraes Rego s/n, CEP 50.670-420, Recife, PE, Brazil*
[c] *Department of Biochemistry, Federal University of São Paulo, Três de Maio street, 100, Vila Clementino, CEP 04044-020, São Paulo, SP, Brazil*

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ABSTRACT

Blood coagulation and platelet-dependent primary homeostasis are important defense mechanisms against bleeding and novel inhibitors have been researched to obtain pharmacological and clinical applications. In this work, the PpyLL, a lectin obtained from Phthirusa pyrifolia, was characterized in terms of its molecular structure and biological functions (anticoagulant, antiplatelet agreggation and hemagglutinating activities) in presence or absence of Gamma radiation exposure. Results revealed a lectin with secondary-structure content by approximately 49% of β-sheet, 20% of β-turn and 31% of disordered structure. Irradiation effect demonstrated possible different sites of function by lectin on anticoagulant and hemagglutinating activities, once a decrease about 80% was observed when compared the activities under 0.5 kGy of exposition to gamma radiation. An emphatic discussion about the use of gamma radiation as a possible modulator of the lectin activity was made, and once the ionizing radiation affected differently the anticoagulation and hemagglutinating activities, we speculated that the results are determined by selective molecular damages in different binding sites. PpyLL biological activities and gamma radiation modulation could be considered for future researches in biomedical field aiming possible medical applications.

1. Introduction

Because of the property to bind carbohydrates, lectins have been used in practical applications and several biological activities as antibacterial, antiviral, imunomodulatory [1], pro-inflammatory [2] hemagglutinating, anticoagulant and antiplatelet aggregation [3]. Specificity with sugars is essentially maintained by particular amino acid residues, which brings a high dependence of the structure-function relationship [1]. Lectins interact with mono, oligosaccharides and with many cellular glycoconjugates, what promotes erythrocyte agglutination and other cell aggregations, for example, in the binding of platelet membranes within other cells [4].

Phthirusa pyrifolia leaf lectin (PpyLL) is produced by the South American hemi-parasitic mistletoe *P. pyrifolia*, a plant well known for the preparation of natural products in the treatment of infections. PpyLL is reported as thermo-stable and with effective antimicrobial activities against pathogens, making it favorable for therapeutic and biotechnological applications [5].

Coagulation cascade comprises the activation of many coagulation factors which promotes the final conversion of fibrinogen to fibrin through the action of thrombin. Lectins has the capability to binding these factors domains and block efficiently the carbohydrate-dependent interactions and affect the amplification of the coagulation cascade [6]. Agents of the coagulation disorders have been focus of many studies and the antithrombotic therapy has evolved by the research of novel elements that activate anticoagulant mechanisms [7].

* Corresponding author at: Departamento de Bioquímica, Centro de Ciências Biológicas, Universidade Federal de Pernambuco (UFPE), Av. Prof. Moraes Rego s/n, CEP 50.670-420 Recife, PE, Brazil.
E-mail addresses: romero.brandao@outlook.pt, romero.bcosta@ufpe.br (R.M.P.B. Costa).

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Lectins are reported to present a wide variety of molecular structure [8] and the number of units per polypeptide chain is one of the main responsible for this variety. Lectin subunits are held together by disulfide bonds, hydrogen bonds and hydrophobic interactions and these interactions have great importance to the molecular stability.

Since tertiary conformation (and consequently the function performed by proteins) is highly dependent on the secondary and primary structure arrangement [9], the protein structural characterization is essential to aid in the identification of protein physico-chemical susceptibilities by alterations in particular amino acid sequences, motifs or residues which sets out the protein fold. Thus, the spectroscopy technique circular dichroism (CD) is useful for biochemistry troubleshooting by: estimating the content of secondary structure [10], providing information about the study of protein molecular structure and to monitoring conformational changes. The study of lectin conformation by CD allows one to understand its character and functionality under several environmental conditions.

Gamma radiation is supposed to damage directly and indirectly the protein structure, not uniformly but in specific points of its sequence [11], resulting in conformational changes in secondary and tertiary structures and impairing biological functions [12]. In the literature, ionizing radiation is applied to: enhance protein properties; to sterilize medical supplies, immobilize enzyme, inhibitor, hormone and other during the treatment of bioactive materials, being the protein the major target to obtain the improvement of the biological material [13]. Food irradiation has been focused on these researches aiming to inactivate undesired components, including lectin functions [14].

The use of ionizing radiation associated with other techniques of obtaining and identification of biomolecules has been since long time reported, so the protein chemistry became important to know the mechanism of enzyme functions, electron migration in proteins and in the sterilization of food and drugs [15]. At the same time, methods to study the molecular structure and the polypeptide selective breakage by radiation can be associated and provide a more detailed functional aspect of the molecules. Structural information such the localization of fragile bonds in the primary structure, detection of a target size by radiation fragmentation or a radiation inactivation size can be obtained by techniques which hits the polypeptide sequence and abolishes selective functions [11].

We studied the gamma radiation influence on the PpyLL anticoagulant, hemagglutining and platelet aggregation activities to understand how the lectin would be susceptible to the ionizing radiation by estimating induced damages. Varying radiation doses could allow one to select lectin functions depending on restrictive injuries in the lectin binding site which prevent the correct carbohydrate linkage. Differences after radiation in the many activities were discussed aiming a possible modulation and application of the PpyLL biological functions.

2. Material and methods

2.1. Chemicals

Sephadex G-75, Superdex-75 (HR/300GL) and ConA lectin were obtained from Sigma (Saint Louis, USA). PT and aPTT reagents (Thromborel S and Dade Actin Activated Cephaloplastin 106 reagent) were purchased from Dade Behring (Marburg, Germany) and stored at 4 °C. All reagents were of analytical grade.

2.2. Isolation of PpyLL lectin through chromatography steps

P. pyrifolia leaves aqueous extract at 10% (w/v) was submitted to a purification process following a previously described protocol [5] to obtain the purified PpyLL lectin. An ÄKTA Avant 25 Explorer-10 system (Pharmacia LKB Biotechnology, Uppsala, Sweden) calibrated with standard proteins was used to chromatograph the samples on a Superdex G-75 HR 10/300 GL column. Lectin was eluted by 0.15 M NaCl solution in water, pH 6.8, followed by lyophilization. Circular dichroism and biological assays were achieved using PpyLL obtained from gel filtration chromatography.

To evaluate the amino-terminal sequence of protein, an aliquot of lyophilized lectin (1.0 mg) was submitted to C_{18} reverse phase chromatography on high performance liquid chromatography-HPLC (Shimadzu LC-10AD-cuz, Kyoto, Japan). The peak was obtained by a nonlinear gradient elution (5%-100%) of acetonitrile 90% in 0.1% TFA in Milli-Q water (stream of 0.7 mL/min). The PpyLL elution was monitored at 215 nm.

2.3. Partial characterization of PpyLL – molecular features

2.3.1. N-terminal sequence

N-terminal amino acid sequence of PpyLL (2 mg) obtained from HPLC, described in the item 2.2, was achieved through denaturating and reducing reaction by addition of 500 mM Tris/HCl buffer, pH 8.5 (200 μL), containing 6 M guadinium HCl, 1.0 mM EDTA and 5 mg dithiothreitol and remained incubated under a nitrogen atmosphere at 37 °C for 3 h. The samples were alkylated, based in the methods of Friedman et al. [16], where 13.4 mg of iodoacetamide dissolved in 100 μL of a 0.5 M NaOH water solution was added and incubated at 37 °C for 2 h under a nitrogen atmosphere. The sample desalination was performed by reverse-phase HPLC. The obtained protein had its N-terminal sequence determined by Edman's degradation [17] in a gas-liquid protein sequencer (PPSQ-23–Shimadzu, Kyoto, Japan). BLAST protein sequence database was used to determine the amino acid sequences.

2.3.2. Circular dichroism spectroscopy for PpyLL characterization

Circular dichroism spectra were recorded on a Jasco J-810 spectropolarimeter (Jasco International Co., Ltd., Tokyo, Japan) equipped with a peltier-type temperature controller and a thermostated cell holder, measurements were made at 25 °C. Samples were applied in a quartz cell (1 cm path) filled with a protein solution concentrated at 0.6 mg/mL in 5 mM sodium phosphate buffer (pH 7.0) was used to submit the samples. PpyLL was analysed at a Far-UV CD spectra (200–240 nm) and each CD spectrum represented the result of three scans, at 50 nm/min, with 1 nm slit width, 1 s response time and 0.5 nm data pitch. CD spectra were background corrected and scaled to molar ellipticity and the CDNN software was used to the secondary structure analysis.

2.3.3. Electrophoresis 2D

GE Life Sciences protocol was used to perform the bidimensional electrophoresis. For the separation through a pH gradient, 40 μg of the protein sample were added to a rehydration buffer (8 M urea; 2% CHAPS; 18 mM DTT; 0.5% IPG buffer pH 3–10 and 0.002% bromophenol blue) for a total volume of 250 μl. Immobiline DryStrips pH 3-10NL (GE Life Sciences, Uppsala, Sweden) was used to load the sample, and was equilibrated in SDS equilibration buffer (6 M urea, 30% glycerol, 2% SDS) for 15 min with 10 mg/mL DTT, and 25 min with 100 mg/mL in the same buffer. The second dimension was performed by SDS-PAGE method in acrylamide gel 15%. The strips were applied onto the gel and sealed with 0.5% agarose and 0.01% bromophenol blue. In the total, the electrophoresis run lasted 20 min at 15 mA per fixed gel and approximately 2 h at 45 mA for approx-