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Purification and Characterization of the Lectin from Taro (*Colocasia esculenta*) and Its Effect on Mouse Splenocyte Proliferation In Vitro and In Vivo

Patrícia Ribeiro Pereira · Eduardo Mere Del Aguila · Maurício Afonso Verícimo · Russolina Benedeta Zingali · Vânia Margaret Flosi Paschoalin · Joab Trajano Silva

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Abstract Lectins are proteins found in a wide range of organisms, with the ability to bind reversibly to specific carbohydrates. They can display important biological activities, such as the activation of the cell cycle in lymphocytes. Storage proteins with lectin activity have been reported in tuberous plant species, such as *Colocasia esculenta*, popularly known as taro. A simple strategy based on Cibacron Blue chromatography was used to purify a 12 kDa polypeptide 1.3-fold, with a recovery of 30 %. The purified protein was identified as tarin by mass spectrometry, which indicated that it was present in G1a/G1d isoforms. Tarin exhibited both agglutinating activity against hamster erythrocytes and mitogenic activity in vitro and in vivo toward mouse splenocytes. Optimum cellular proliferation in vitro was achieved by 625 ng of the crude extract or 500 ng of the purified tarin. Total mouse splenocyte proliferation measured after 5 days of intraperitoneal inoculation of purified tarin was increased 3.3-fold in comparison to the control group. Half of the proliferating cells were identified as B lymphocytes by flow cytometry. These results show that this is an efficient and simple strategy to purify tarin and aid in establishing this protein as a new therapeutic drug, able to promote cell proliferation in a murine model.

Keywords *Colocasia esculenta* · Tarin · Purification · Mitogenic and hemagglutinating activities · MALDI-TOF-TOF analysis

Abbreviations

[3H]-timidine	(Tritiated)-thymidine
Anti-IgM	Anti-immunoglobulin M antibody
BSA	Bovine serum albumin
Con A	Concanavalin A
FACS	Fluorescence-activated cell sorter
FCS	Fetal calf serum
FITC	Fluorescein isothiocyanate
GNA	*Galanthus nivalis* agglutinin
HIV	Human immunodeficiency virus
MALDI	Matrix assisted laser desorption ionization
PBS	Phosphate buffered saline
PE	Phycoerythrin
SDS-PAGE	Sodium dodecyl sulfate-polyacrylamide gel electrophoresis
TOF	Time of flight

1 Introduction

Plant lectins comprise a group of proteins with at least one non-catalytic domain that binds specifically and reversibly to simple or complex carbohydrates [1, 2] through hydrogen bonds or Van Der Waals interactions, including or not

P. R. Pereira · E. M. Del Aguila · V. M. F. Paschoalin (✉) · J. T. Silva
Instituto de Química, Universidade Federal do Rio de Janeiro (UFRJ), Avenida Athos da Silveira Ramos, 149 Bloco A, sala 545, Cidade Universitária, Rio de Janeiro, RJ CEP 21941-909, Brazil
e-mail: paschv@iq.ufrj.br

M. A. Verícimo
Instituto de Biologia, Universidade Federal Fluminense (UFF), Alameda Barros Terra S/N, Campus Universitário do Valonguinho, Laboratório de Imunologia das Doenças Infecciosas e Granulomatosas, 3° andar, Centro, Niteroi, RJ 24020-141, Brazil

R. B. Zingali
Instituto de Bioquímica Médica, Universidade Federal do Rio de Janeiro (UFRJ), Av. Carlos Chagas Filho, 373. CCS Bloco H, 2° andar, sala 08. Ilha do Fundão, Rio de Janeiro, RJ 21941-590, Brazil

agglutinating activity or glycoconjugate precipitation [3]. Lectins are ubiquitous in almost all living beings, from the animal and plant kingdoms, bacteria, protozoa, viruses, fungi and algae, [2] and have different functions, according to where they are found [4]. As expected for a widely distributed protein, lectins have variable structural characteristics such as size, molecular organization and composition of the binding sites [5, 6].

Plants are a rich source of lectins, in particular in their storage organs such as seeds, tubers, bulbs and rhizomes [4]. Although plant lectins have specificity toward monosaccharides, they bind with higher affinity to the more complex glycans that are found in animals and humans but not in plants [1]. They are capable of recognizing glycoconjugates present on the microorganism surface or in the intestinal tract of insects and herbivorous animals, indicating that they are part of defense mechanisms in plants [1, 7].

Taro (*Colocasia esculenta*) is a tuberous plant originating from Asia that grows in tropical and subtropical climates, where it is widely used for human consumption as a supplementary food source [8]. Taro extracts have been reported to have important pharmacological properties including anti-inflammatory, anti-cancer, antifungal, anti-viral and insecticidal activities [9]. Evidence shows that the lectin present in taro extracts contributes to some of these biological activities [10, 11]. Some plant lectins interact with carbohydrates from the surface of hematopoietic cells, leading to cell proliferation. These proteins, known as mitogenic lectin, have been fundamental in human immunological studies because some of them can activate lymphocytes [12]. Hence, they can be used to evaluate the immune status of patients with various diseases including AIDS [13] or affected by immunosuppressive and immunotherapeutic treatments [14]. Mitogenic lectins are also used in genetic studies for different purposes, such as karyotyping [15] and the determination of sex or chromosome aberrations [16]. These characteristics of mitogenic lectins make them useful for curative purposes, providing protection and recovery from immunosuppressive effects caused by tumors and infections [17]. Additionally, they are valuable tools to elucidate the biochemical mechanism involved in the activation of lymphocytes and proliferation of other immune cells [5].

Here, we report the purification of a 12 kDa lectin-like protein, identified as tarin, which is able to induce proliferation of mice B lymphocytes in vitro and in vivo.

2 Materials and Methods

2.1 Biological Material

Tubers of taro (*C. esculenta*) were purchased from a local store in Niterói city, Brazil. Male inbred C57Bl/6 mice were obtained from the Laboratory Animal Center (NAL) at the Universidade Federal Fluminense (UFF). Hamsters were purchased from a local pet shop in Niterói. The experiments with animals were approved by the Universidade Federal Fluminense Ethics Committee under number 0019-08.

2.2 Crude Extract Preparation

Crude extract was obtained according to the protocol described by Roy et al. [18]. One hundred grams of taro tubers was homogenized in 0.2 M NaCl containing 1 g/L ascorbic acid (10 mL/g fresh weight) at pH 7.0 in a Waring blender. The homogenate was filtered through cheesecloth and centrifuged at $3{,}000\times g$ for 10 min. $CaCl_2$ (20 mM) was added to the supernatant, the pH was adjusted to 9.0, and the solution was stored overnight at 8 °C. After this period, it was centrifuged at $3{,}000\times g$ for 10 min, the supernatant was collected, the pH was readjusted to 4.0 and the sample was centrifuged at $3{,}000\times g$ for 10 min. The clear supernatant was allowed to stand overnight in an ice bath, and after adjusting the pH to 7.5, the precipitate was removed by centrifugation at $9{,}000\times g$ for 20 min. The crude extract was stored at −20 °C. The protein concentration of the crude and purified fractions was estimated by the method of Lowry [19], using bovine serum albumin (BSA) as a standard.

2.3 SDS-PAGE Analysis

Homogenous polyacrylamide gels at 15 % and discontinuous buffer systems under denaturing and reducing conditions were set up according to Laemmli [20]. Protein bands were observed by staining with Coomassie® Brillant Blue G-250 Ultra Pure (USB Corporation).

2.4 Lectin-Like Protein Purification

Six milliliters of crude extract (1.75 mg/mL) was chromatographed on a Cibacron Blue 3GA column (1.0 × 6.5 cm) previously equilibrated with 10 mM Tris–HCl pH 7.5. After sample application, unbound proteins were washed with the same buffer and the bound lectin-like protein was eluted with 0.4 M NaCl in the same buffer. Flow rate was maintained at 0.7 mL/min, and 2 mL fractions were collected.

2.5 Identification of the Lectin-Like Protein

The purified polypeptide chain was identified by means of mass spectrometry (MALDI-TOF-TOF 4700 Proteomics Analyzer, Applied Biosystems). A fragment of the gel containing the purified protein was washed three times with 25 mM ammonium bicarbonate/acetonitrile 1:1 (v/v). After

washing, the sample was covered by 100 % acetonitrile which was removed after 10 min, and then the digestion buffer containing 10 μg/mL trypsin in 25 mM ammonium bicarbonate was added, followed by incubation at 37 °C for 18 h. The masses of the peptide fragments were determined by mass spectrometry. Selected peptides were fragmented in the second dimension, and the protein was identified using the MASCOT search engine (http://mas8.lnls.br/mascot).

2.6 Agglutination Assay

Hemagglutination assays of the crude and purified fractions were carried out using hamster erythrocytes in a 96-well microtiter plate, with increasing amounts of protein in crude extracts (from about 48 ng to100 μg) or purified fractions (from 4.8 ng to 10 μg). Samples were serially diluted (1/2) in phosphate buffered saline (PBS) along the plate in a volume of 50 μL and mixed with an equal volume (50 μL) of 1 % red blood cells (RBCs) suspended in PBS. Plates were incubated at 37 °C for 1 h or until the control assays (erythrocyte suspension only) were completely sedimented.

2.7 Proliferation Assay

Increasing amounts of protein in crude extracts (from 9.7 ng to 20.0 μg) or purified fractions (from 1.9 ng to 4.0 μg) were added to 200 μL of RPMI-1640 medium (supplemented with 10 % fetal bovine serum, 2 mM L-glutamine, 5×10^{-5} M β-mercaptoethanol and 20 μg/mL gentamicin). In culture medium containing crude taro extracts or purified tarin, splenocytes from a C57Bl/6 mouse at a concentration of 4×10^5 cells/mL were cultured for 72 h at 37 °C in a 5 % CO_2 atmosphere. Cells were pulsed with [^{3}H]-thymidine (0.5 mCi/well) for 16 h. Assays were performed in three independent experiments.

2.8 Mitogenic Activity (In Vivo) Evaluation

Male C57Bl/6 mice were treated with the purified or crude fractions of tarin. Eight animals were treated by intraperitoneal administration of saline (1 mL) containing the purified fraction (0.1 mg) or the crude extract (1 mg). Control animals received 1 mL of saline. On day 5 or 10 after inoculation, mouse spleens were removed for later analysis.

2.9 Preparation of Cell Suspension

Spleens were homogenized in cold PBS. Cell suspensions were washed in PBS by centrifugation at 258×*g* at 4 °C for 7 min and the supernatants were discarded. Pellet cells were submitted to osmotic shock for 10–20 s under manual agitation to eliminate erythrocytes. The erythrocyte-free cell suspension was again washed by centrifugation and the resulting pellet was suspended in 2 mL of cold PBS.

2.10 Cell Counting

Splenocytes were diluted in Türk solution (1/200) and the total number of cells was counted in a Neubauer chamber with the aid of optical microscopy.

2.11 FACS Staining

One million splenocytes from each mouse were treated with PBS supplemented with normal 3 % mouse serum to block non-specific binding of antibodies. After 20 min at 4 °C, cells were collected by centrifugation (560×*g* for 7 min at 4 °C) and stained with anti-IgM FITC (Southern Biotechnology), anti-B220 PE (Pharmingen). Then, PBS containing 0.001 % sodium azide and 3 % fetal calf serum (FCS) was added to the cells. After 40 min incubation in the dark at 4 °C, the cells were washed with cold PBS and then fixed in 1 % formol. Fluorescence intensity was detected in a Flow Cytometer (FACScalibur, Becton–Dickinson), where a gate containing only lymphocytes was selected based on their size and granularity patterns; the results were quantified using WinMDI 2.8 software.

2.12 Statistical Analysis

The total number of splenocytes recovered and the amounts of $B220^+IgM^+$ lymphocytes were compared using a variance analysis (ANOVA) followed by a multiple comparisons analysis by the Tukey method [21]. The results were considered significant if $p < 0.001$ or $p < 0.01$, analyzed using InStat software (GraphPad, San Diego, CA).

3 Results

3.1 Purification and Identification of a Lectin-Like Protein

SDS-PAGE analysis (15 %) of the crude extract obtained from taro tubers revealed the presence of three major groups of proteins with apparent molecular masses of about 60, 25 and 12 kDa (Fig. 1, lane 1). Another polypeptide chain of less than 10 kDa molecular mass was also observed (data not shown), which may correspond to a proteolytic form, since the intensity of this band increased during the storage period.

Most of the protein content from the crude extract was not observed after the Cibacron Blue dye affinity

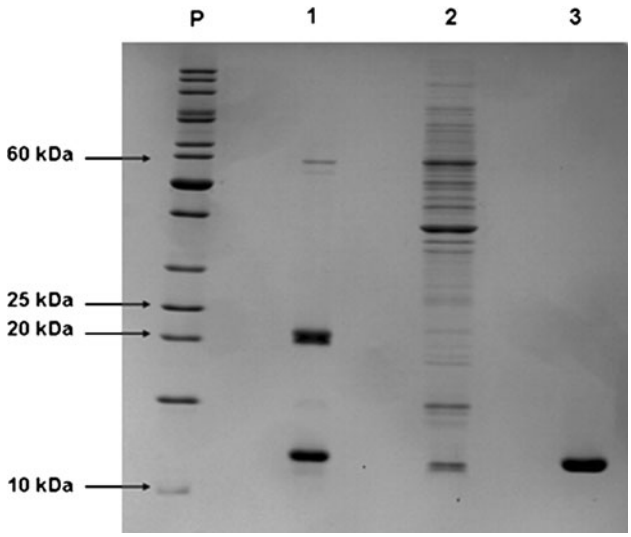

Fig. 1 SDS-PAGE (15 %) analysis of tarin. A crude extract from taro tubers was fractionated on a Cibacron Blue chromatographic column, and the collected fractions were analyzed on a 15 % SDS-PAGE gel. *Lane P*—molecular mass marker (PageRuler^{TM} Unstained Protein Ladder, Thermo Scientific); *lane 1*—10 µL of the crude extract of *C. esculenta* (0.95 mg/mL); *lane 2*—5 µL of pooled fractions (3 mg/mL) from washing step; *lane 3*—10 µL of the fraction (0.45 mg/mL) of adsorbed-matrix proteins eluted in the presence of 0.4 M NaCl

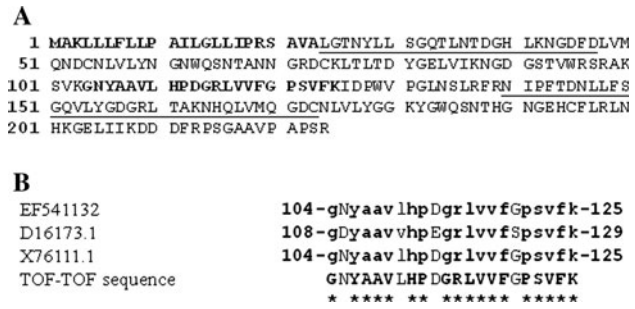

Fig. 2 Identification of the purified protein by mass spectrometry (MALDI-TOF-TOF). In *bold letters*, the signal peptide; in *gray*, the peptides GNYAAVLHPDGR (104-115) and LVVFGPSVFK (116-125). The *underlined regions* correspond to Edman degradation of the N-terminal of subunits G1d (25-LGTNYLLSGQTLNTDGHLKNG DFD-47) and G1a (140-NIPFTDNLLFSGQVLYGDGRLTAKNHQ LVMQDGC-173) reported by De Castro et al. [8]. This sequence corresponds to GenBank **ABQ32294.1** (**a**). Both peptides (104-GNYAAVLHPDGR-115 and 116-LVVFGPSVFK-125) were aligned with homologous sequences of tarin deposited in GenBank (**EF541132**, **D16173.1** and **X76111.1**). Three variable positions were detected in this sequence (N105D; D113E and G120S) (**b**)

chromatography, and consequently the proteins must be present in the fractions corresponding to the wash step from the chromatographic matrix (Fig. 1, lane 2). The 12 kDa polypeptide observed in the crude extract (Fig. 1, lane 1) was adsorbed to the matrix. The matrix-bound polypeptides were eluted by 0.4 M NaCl, and a protein of 12 kDa (Fig. 1, lane 3) was purified 1.3-fold, with 30 % recovery (Table 1).

The purified protein was digested by trypsin and the resultant peptides were analyzed by mass spectrometry (MALDI-TOF-TOF). Two peptides showed monoisotopic masses of 1091.6379 (LVVFGPSVFK) and 1268.6262 (GNYAAVLHPDGR) (Fig. 2a). These two peptides were used to search for homologue proteins in GenBank through the BLASTP program [22, 23]. The first peptide was found in the 116–125 region of tarin from *C. esculenta* (GenBank **ABQ32294.1**), while the second was found in the 104–115 region of tarin and in the 105–116 region of a mannose-binding lectin from *Typhonium divaricatum* (GenBank **ABM68041.1**). These results unequivocally identified the purified protein as *C. esculenta* tarin.

Three independent entries in GenBank report tarin sequences (**EF541132**, **D16173.1** and **X76111.1**), which have a peptide sequence very similar to that obtained by mass spectrometry. They differ in a few amino acids, N105D, D113E and G120S, indicating the presence of different isoforms of this protein (Fig. 2b).

3.2 Tarin Characterization

Colocasia esculenta tarin showed agglutinating activity against rabbit erythrocytes but not against human erythrocytes, independent of blood type [18, 24]. Both the crude extract and purified tarin showed agglutinating activity against hamster erythrocytes. The minimum agglutinating activities of the crude extract and the purified tarin were achieved using 16.4 and 12.5 µg/mL, respectively. By the same assay, the agglutinating activity against rabbit

Table 1 Purification of the putative lectin from *C. esculenta*

Purification step	Protein (mg/mL)	Volume (mL)	Total protein (mg)	Total activity (UH)	Specific activity (UH/mg)	Recover (%)	Purification fold
Crude extract	2.1	5.0	10.5	6,402	610	100	1
Cibacron Blue chromatography	0.2	12.0	2.4	1,920	800	30	1.3

UH—hemagglutinating unit, corresponds to the minimum quantity of protein capable of inducing agglutination; UH/mg corresponds to the amount of hemagglutinating units per milligram of protein

erythrocytes showed that the minimum agglutinating activities were lower for the crude extract (4.1 μg/mL) and for the purified tarin (3.1 μg/mL), in agreement with previous results [24].

3.3 In Vitro Proliferation Assay in the Presence of Tarin

Preliminary results showed that the crude extract was capable of stimulating the proliferation of mice splenocytes. In order to assess if cell proliferation could be attributed to tarin or to another component in the extract, splenocytes were cultured in the presence of increasing quantities of the purified tarin (from 9.5 ng/mL to 20.0 μg/mL) or of the crude extract (from 48.5 ng/mL to 100 μg/mL). The proliferative activities proved to be dependent on the amount of protein added to the assay. Larger amounts of protein were not able to induce cellular proliferation or even cause inhibition. Indeed, a high amount of lectin is known to inhibit cell proliferation [25]. Maximum cellular proliferation was achieved with 3.12 μg/mL of the crude extract or 2.5 μg/mL of purified tarin, although a small amount of cellular proliferation was detected with 0.39 μg/mL of the crude extract (data not shown) and 0.16 μg/mL of the purified tarin (Fig. 3).

3.4 In Vivo Effect of Lectin on Mice Splenocytes

To investigate if the tarin proliferation effect in cell culture could be reproduced in vivo, C57Bl/6 mice were treated with crude extract and purified tarin through intraperitoneal administration. Five and ten days after inoculation, mouse spleens were removed and the number of splenocytes was counted. The results revealed that the mitogenic effect of tarin observed in vitro, was also observed in vivo (Fig. 4a). The total number of recovered splenocytes from each mouse group increased in both groups treated with the purified tarin ($222.66 \pm 46.36 \times 10^6$ cells) or with the crude extract ($487 \pm 133.96 \times 10^6$ cells) in comparison to the control group ($68 \pm 15.66 \times 10^6$ cells) (Fig. 4a), corresponding to increases of 3.3- and 7.2-fold respectively.

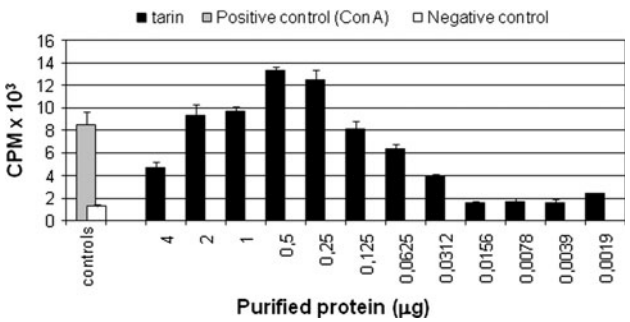

Fig. 3 In vitro effect of purified tarin on cell proliferation. The cells (4×10^5) were cultured in 200 μL of RPMI medium containing increasing amounts of the purified protein (from 1.9 ng to 4.0 μg), compared to those without stimulus (control group) or cultured in the presence of Concanavalin A (positive control groups). Cells were pulsed with [H^3]-thymidine for 16 h before the culture was ended. Proliferative activity is represented by scintillations per minute (CPM). Values correspond to means ± SD from three independent experiments

3.5 Phenotypic Analysis of the Mouse Splenocytes After Tarin Treatment

To identify which splenocyte cell lines were proliferating, cells were stained with the monoclonal antibodies anti-B220/PE and anti-IgM/FITC (B lymphocytes). Five days after inoculation of the purified tarin ($107.92 \pm 24.63 \times 10^6$ cells) or crude extract ($238.85 \pm 59.79 \times 10^6$ cells), both proved to be capable of inducing B lymphocyte proliferation (Fig. 4b) in comparison to the control group ($26.23 \pm 7.56 \times 10^6$ cells). These results indicated that the mitogenic effect of tarin may be a physiological cell effect, and that this in vivo proliferation is directed to B lymphocytes.

4 Discussion

Many lectins have been isolated from members of the family Araceae, including taro (*C. esculenta*). These proteins are characterized by the ability to bind specifically to mannose, and are currently referred to as *Galanthus nivalis* agglutinin (GNA)-related lectins. They have attracted increasing attention in recent years because of their remarkable biological properties, making them a good prospect for biotechnological applications [26].

According to Bezerra et al. [27], taro corms are composed of four major protein families, two albumins, A1 (with molecular mass of 12–14 kDa) and A2 (with molecular mass of 55–66 kDa); and two globulins, G1 (with molecular mass of about 14 kDa) and G2 (with molecular mass of about 22 kDa). The G2 globulin family is composed of two protein bands of 24 kDa (G2a) and 22 kDa (G2b) [28], whereas G1 is composed of a large number of isoforms of 12 kDa with isoelectric points near 7.5 [29, 30]. Previous studies have shown that those polypeptides are present exclusively in tubers from *C. esculenta*, and have not been found in other plant organs [31].

Hirai et al. [31] observed that each group of proteins was composed of two or three bands, although, in our preparation (Fig. 1, lane 1) this band distribution was not observed. The method of extraction used in the present study included precipitation steps caused by pH variation, which may account for the absence of some protein bands in our preparation.

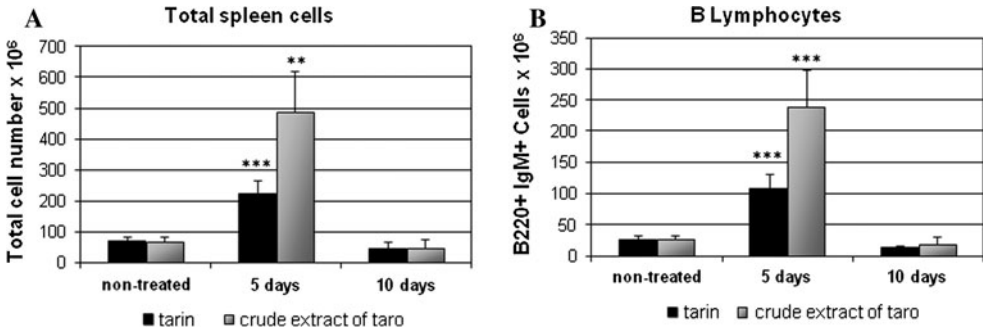

Fig. 4 In vivo effect of purified tarin and crude extract on cell proliferation. C57Bl/6 mice received intraperitoneally, 1 mg of the crude extract of taro or 0.1 mg of the purified tarin diluted in 1 mL of saline, or 1 mL of saline solution (non-treated group). Each group was composed of 4 animals. At days 5 and 10, mouse spleens were removed and the total number of cells was counted (**a**). Recovered cells from the mouse groups were stained with monoclonal antibodies anti-B220 and anti-IgM for B lymphocytes, and analyzed in a Flow Cytometer (**b**). Results are expressed as mean ± SD. *Asterisks* indicate significance level $p < 0.001$ (***) and $p < 0.01$ (**) in comparison to the non-treated group

The polypeptide bands of 25 and 12 kDa were previously identified and their genes were cloned [27, 31]. Additionally, de Castro et al. [8] found that there is no amino acid homology between the N-terminals of the two proteins, indicating that they are not related to each other. Amino acid sequencing of the 25 kDa polypeptide showed that it is homologous to the trypsin inhibitor family found in soybeans, winged beans, sweet potato and barley [31].

The cloning of the sequence encoding the 12 kDa polypeptide showed that this protein is synthesized as a precursor polypeptide of about 28 kDa, which is processed into two subunits of about 12 kDa [27, 31], in agreement with the results shown in the SDS-PAGE (Fig. 1). The amino acid sequence deduced showed that the protein is homologous to mannose-binding lectins. Further studies confirmed that this protein exhibits hemagglutinating activity, confirming its identity as a lectin [24]. Based on the above information, this study focused on the taro lectin, which corresponds to the 12 kDa polypeptide band observed in denaturing polyacrylamide gels.

Here, the 12-kDa protein (G1 globulin), later named tarin since it is encoded by the *Tar1* gene [27], was purified from the tubers of *C. esculenta*, using a rapid, single-step purification procedure which includes an affinity chromatographic column.

A semi-quantitative evaluation of the polypeptide band intensities on the SDS-PAGE was performed by densitometric analysis using the GelAnalyzer 2010 software (www.gelanalyzer.com). Based on the amount of protein loaded on the gel, the 12 kDa polypeptide band, following Cibacron Blue chromatographic column purification step, is more than 90 % pure (Fig. 1). However, based on the hemagglutinating activity, tarin showed 1.3-fold purification with 30 % recovery (Table 1). The apparent discrepancy between the SDS-PAGE profile and the hemagglutinating activity may result from an underestimation of the biological activity of the purified protein. The purification step could cause a modification of the protein environment that protects its biological activity, and/or even cause the loss of an essential cofactor for the hemagglutinating activity of tarin. Indeed, it is well known that some lectins require divalent cations to agglutinate erythrocytes [32–35].

Another point to consider is that during tarin purification on Cibacron Blue, the 25 kDa polypeptide chain is removed. The clearance of this inhibitor of protease activity may increase tarin proteolysis, generating low-molecular-mass polypeptides and consequently reducing the hemagglutinating activity.

Previous studies that reported the purification of tarin from *C. esculenta* used time-consuming methods involving chromatographic columns following precipitation by ammonium sulfate [31], or they employed expensive affinity columns with asialofetuin-linked columns, which are not easily available [24].

De Castro et al. [8] observed, by two-dimensional electrophoresis, the presence of four isoforms of the G1 globulin from taro and termed them G1a, G1b, G1c and G1d. Microsequencing studies revealed homology between the G1a and G1c isoforms and between the G1b and G1d isoforms. Comparing the sequencing data from mass spectrometry with the sequences in GenBank (Fig. 2b) shows that there are three variable residues in that region: N105D, D113E and G120S. The peptides containing amino acid residues N105, D113 and G120, present in the sequences **EF541132** and **X76111.1**, are part of the gene that contains the G1a and G1d isoforms characterized by De Castro et al. [8]; while the peptide containing the amino acid residues D105, E113 and S120 within the sequence **D16173.1**, deposited by Hirai et al. [31], is part of the gene that encodes the G1b and G1c isoforms. Therefore, it seems that two distinct genes can encode tarin, and the

resulting polypeptides differ in a few amino acid residues. The isoform purified in this study seems to correspond to that described in the sequences **EF541132** and **X76111.1.**

The purified tarin is able to stimulate, both in vitro and in vivo, total splenocyte proliferation which is directed to B lymphocytes (Figs. 3, 4), suggesting a potential to mimic cytokine activity. The proliferation effect described in this study is similar to proliferation curves found using other mitogenic lectins, such as Concanavalin A and phytohemagglutinin, with optimal doses corresponding to intermediate quantities of the crude extract or purified tarin [25]. The crude extract was more effective than the purified tarin in triggering proliferation (Fig. 4), but it must be considered that tarin comprises 40 % of the total proteins present in the crude extract [31]. An alternative that should also be considered is that an enhancing factor for tarin activity could have been eliminated during purification, causing underestimation of the proliferation activity in vivo.

Root crops such as taro are particularly interesting since they are widely available sources of natural mimetic molecules [36]. Tarin can be considered as an alternative to other commercially available lectins. This protein could be used as a tool to study lymphocyte transformation as a model of antigenic activity, as well as initiation of cellular growth and division; and to assess the immune status of an individual suffering from immune disorders [25, 37, 38]. Several agents derived from plants and food have demonstrable preventive or therapeutic activity by multiple mechanisms, including as a stimulus to recover the immune-system status from impairment caused by pathological conditions or medical treatments [36, 39–41].

Plant proteins offer a good opportunity to apply novel molecules with significant potential as new therapeutic drugs. Tarin can be safely produced and is readily available, particularly in tropical and subtropical regions with suitable climate and soil conditions.

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