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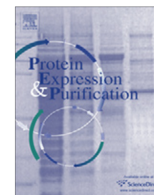

Expression of frutalin, an α-D-galactose-binding jacalin-related lectin, in the yeast *Pichia pastoris*

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ABSTRACT

Frutalin is an α-D-galactose-binding lectin expressed in breadfruit seeds. Its isolation from plant is time-consuming and results in a heterogeneous mixture of different lectin isoforms. In order to improve and facilitate the availability of the breadfruit lectin, we cloned an optimised codifying frutalin mature sequence into the pPICZαA expression vector. This expression vector, designed for protein expression in the methylotrophic yeast *Pichia pastoris*, contains the *Saccharomyces* α-factor preprosequence to direct recombinant proteins into the secretory pathway. Soluble recombinant frutalin was detected in the culture supernatants and recognised by native frutalin antibody. Approximately 18–20 mg of recombinant lectin per litre medium was obtained from a typical small scale methanol-induced culture purified by size-exclusion chromatography. SDS–PAGE and Edman degradation analysis revealed that frutalin was expressed as a single chain protein since the four amino-acid linker peptide “T-S-S-N”, which connects α and β chains, was not cleaved. In addition, incomplete processing of the signal sequence resulted in recombinant frutalin with one Glu-Ala N-terminal repeat derived from the α-factor prosequence. Endoglycosidase treatment and SDS–PAGE analysis revealed that the recombinant frutalin was partly *N*-glycosylated. Further characterisation of the recombinant lectin revealed that it specifically binds to the monosaccharide Me-α-galactose presenting, nevertheless, lesser affinity than the native frutalin. Recombinant frutalin eluted from a size-exclusion chromatography column with a molecular mass of about 62–64 kDa, suggesting a tetrameric structure, however it did not agglutinate rabbit erythrocytes as native frutalin does. This work shows that the galactose-binding jacalin-related lectins four amino-acid linker peptide “T-S-S-N” does not undergo any proteolytic cleavage in the yeast *P. pastoris* and also that linker cleavage might not be essential for lectin sugar specificity.

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The jacalin-related lectins family derives its name from jacalin, the trivial name of the galactose-binding lectin isolated from jackfruit seeds (*Artocarpus integrifolia*) [1,2]. This family comprises all plant lectins that are structurally and evolutionarily related with jacalin. According to their specificity, the jacalin-related lectins family is subdivided into mannose-specific and galactose-specific lectins. The specificity of galactose-specific lectins is thought to be determined by the size and conformation of the carbohydrate-binding site, which is more extended for galactose-specific lectins than for mannose-specific lectins due to the protomer cleavage into two polypeptide chains (β and α chains) by a tetrapeptide linker post-translational excision [3,4].

The aim of this work is the production and purification of the α-D-galactose-binding jacalin-related lectin from *Artocarpus incisa* seeds—frutalin—in the methylotrophic yeast *Pichia pastoris*. Many structural and functional similarities between jacalin lectin and a lectin from the breadfruit seeds (*Artocarpus incisa* or *Artocarpus altilis*) were previously found [5]. This lectin was further isolated and named frutalin [6]. Frutalin was characterised in physical–chemical and immunologic studies [7–9] and successfully used in immunohistochemical studies on the recognition of cancer metastases, specifically those of breast and thyroid gland (unpublished results). The availability of frutalin in large scale will be necessary to facilitate its application on cancer diagnosis and therapy. One way to obtain large amounts of frutalin with defined amino-acid sequence is through its heterologous expression in microorganisms. Moreover, the expression and production of pure lectin isoforms may contribute to elucidate lectin sugar-binding specificity.

The methylotrophic yeast *P. pastoris* was chosen as expression system due to its well-known expression methodologies, especially for the secretion of recombinant proteins [10,11]. On the other hand, being an eukaryotic system, it is capable of post-translational modifications such as glycosylation. Frutalin has highly glycosylated isoforms, and the presence or absence of the carbohydrate side chains might have significant effects on its functional and/or physical properties.

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In this work, recombinant frutalin was expressed in *P. pastoris* KM71H and purified by size-exclusion chromatography. The molecular and biological characterisation of the recombinant frutalin produced is investigated and compared to that of the native plant lectin. To our knowledge, this is the first time that a galactose-binding jacalin-related lectin was cloned and expressed in an eukaryotic expression system.

Materials and methods

Strains and plasmid

Escherichia coli TOP10 (Invitrogen) was used for plasmid maintenance and construction. The methylotrophic *P. pastoris* KM71H yeast strain (Invitrogen) was used as recipient strain for transformation and subsequent expression of the recombinant frutalin. Vector pPICZαA (Invitrogen) was used as carrier vector for integration of the optimised frutalin gene into the *AOX*1 loci of the yeast genome.

Plasmid construction

The base sequence that codifies the frutalin heavy chain (α chain) was synthesised *in vitro*, based on the amino-acid sequence of frutalin, and also taking into consideration the preferential use codons for *P. pastoris* (Genscript Corporation, Piscataway, NJ, USA). The heavy (α) and light (β) frutalin chains, connected by the "T-S-S-N" linker, were obtained by PCR. The amino-acid linker sequence was obtained by cDNA frutalin synthesis and sequencing (data not shown). The design of the primers was based on the amino-acid sequence determined for frutalin, and it also took into consideration the preferential use codons for *P. pastoris*. A forward "Megaprimer" containing the β chain, the linker and part of the α chain N-terminal (5′ CTC**GAATTC**AATCAACAATCTGGTAA ATCTCAAACTGTTATTGTTGGTCCATGGGGTGCTAAGGTTTCTACTAG CTCCAATGGTAAAGCTTTCGACGATG 3′) and a reverse primer containing part of the α chain C-terminal (5′ CAC**TCTAGA**TT<u>TCA</u>CA-AAGACAAGTAC 3′) were used. The restriction sites EcoRI and XbaI were included in the forward and reverse primers, respectively (in bold), and a stop codon was included in the reverse primer (underlined) to express recombinant frutalin with its native C-terminal. The PCR conditions were: an initial denaturation step at 95 °C for 2 min, followed by 30 cycles of amplification with 45 s of denaturation at 95 °C, 45 s of annealing at 50 °C and 1 min of elongation at 72 °C. Finally, an elongation step at 72 °C for 10 min was conducted. Plasmid pUC57 containing optimised codifying frutalin α chain sequence was used as DNA template and 0.5 μl of Vent DNA polymerase 5 U/l (Stratagene) was employed. After amplification, the 0.5 kb PCR product was purified from 1.7% (w/v) agarose gel, using the Qiaquick Gel Extraction Kit (QIAgen). The purified PCR product was digested with EcoRI and XbaI (Fermentas) and purified once again. Optimised frutalin sequence was then cloned into the multiple cloning site of the pPICZαA expression vector, which had also been previously digested with the same enzymes and purified from agarose gel. This reaction was carried out by incubating the DNA fragments overnight at 4 °C with T4 DNA ligase (Promega). LB plates (0.5% NaCl, 0.5% yeast extract, 2% tryptone and 2% agar, pH 7.5), supplemented with 75 μg/ml of Zeocin (Invitrogen) and incubated at 37 °C, were used for recombinants selection.

Pichia transformation and recombinant frutalin expression

The constructed plasmid, prepared from *E. coli* using the Qiagen Plasmid Midi Kit (QIAgen), was linearised with SacI and transformed into *P. pastoris* KM71H strain by electroporation, using Gene Pulse X-Cell (Bio-Rad). Transformation protocol was performed as described in the manual of the Easy-Select *Pichia* Expression kit (Invitrogen). Transformants were selected on YPD–sorbitol plates (2% glucose, 2% peptone, 1% yeast extract and 1 M sorbitol) containing 100 μg/ml of Zeocin and incubated at 30 °C. Insertions were confirmed by "colony PCR". A very small amount of cells was picked up with a sterile toothpick and placed into a sterile PCR tube. The tubes were warmed twice in a microwave at 900 W for 45 s and chilled on ice. Finally, the Master mix, containing frutalin specific primers, was distributed by the tubes. The PCR conditions used were the same as described above ("Plasmid construction"). Cells of *P. pastoris* KM71H strain containing empty pPICZαA were used as PCR negative control. Positive PCR transformants were picked up in YPD plates containing increasing concentrations of Zeocin (100–1000 μg/ml).

The transformants with the highest resistance to Zeocin were grown overnight at 30 °C, shaken at 250 rpm in 100 ml of buffered minimal glycerol medium—BMG—(1% glycerol, 100 mM potassium phosphate pH 6.0, 1.34% yeast nitrogen base, 4×10^{-5}% biotin), centrifuged at 4000*g* for 10 min at room temperature, and suspended into 50 ml of fresh buffered minimal methanol medium—BMM—(same composition as BMG but glycerol is replaced with 0.5% methanol.) Inducible cultures were incubated at 15 °C, shaken at 300 rpm, and fresh methanol was added daily to a final concentration of 0.5% (v/v) during the 4 days of induction. To overcome oxygen limitations, induction was carried out in 500 ml baffled flasks filled with 50 ml of culture medium and covered with two layers of sterile gauze.

Native frutalin purification

Native frutalin was extracted from breadfruit seeds (*A. incisa*) and purified by affinity chromatography on a cross-linked *Adenanthera pavonina* galactomannan column, as described earlier [6].

Recombinant frutalin purification

The supernatants from small scale methanol-induced cultures were separated from yeast cells by centrifugation (10 min at 4000*g* and 4 °C). The pH of the supernatants was increased to 7.5 by adding 10 N NaOH to precipitate salts, which were removed by centrifuging twice for 10 min at 4000*g* and 4 °C. Then, supernatants were filtered through 0.22 μm pore size filters, concentrated and washed with PBS buffer (8 g/l NaCl, 0.2 g/l KCl, 2.68 g/l $Na_2HPO_4 \cdot 7H_2O$ and 0.24 g/l KH_2PO_4, pH 7.4), to a final volume of 1–1.5 ml in 10 kDa Amicon tubes (Millipore). Treated supernatants were analysed by SDS–PAGE and Western blot for the presence of the recombinant lectin and loaded on a Sephacryl® S-100 HR column using a FPLC system (Pharmacia Biotechnology). The equilibration and the elution of the column were done with PBS at a flow rate of 1 ml/min. The presence and purity of the recombinant lectin in the pooled fractions were checked by SDS–PAGE followed by silver nitrate staining. Fractions containing pure recombinant frutalin were concentrated in 10 kDa Amicon tubes and loaded again on the size-exclusion column to estimate the recombinant lectin molecular mass. Native frutalin and BSA were used as molecular mass standards proteins.

Deglycosylation of recombinant frutalin with Endoglycosidase H

To determine if the recombinant frutalin is *N*-glycosylated, the protein was digested with Endoglycosidase H according to the manufacturer's instructions (New England Biolabs). The digested protein was analysed by SDS–PAGE.

SDS–PAGE and Western blot analyses

Proteins were analysed using denaturing SDS–PAGE with 12% gels, as described by Laemmli [12]. Bands were visualised by staining with Coomassie Brilliant Blue R250 or with silver nitrate (Sigma). Proteins were transferred from gels to a PVDF membrane (Millipore) by using a semi-dry blot apparatus (Bio-Rad), which was followed by immunodetection. The membrane was incubated for 1 h in PBS containing 5% (w/v) non-fat milk powder (Molico) and 0.01% Tween 20 (Sigma). Polyclonal frutalin antibody (produced by inoculating New Zealand rabbits with frutalin) was diluted to 50 μg/ml with PBS containing 1% (w/v) non-fat milk powder and 0.01% Tween 20. The secondary antibody used was "anti-rabbit IgG peroxidase" (Sigma), diluted 1:500 with PBS containing 0.01% Tween 20. The membrane was washed once for 15 min and twice for 5 min with primary antibody dilution solution and then incubated with the primary antibody for 1 h. It was then washed once for 15 min and twice for 5 min with secondary antibody dilution solution followed by secondary antibody incubation for 30 min. Finally, the membrane was washed once for 15 min and twice for 5 min with PBS and incubated briefly with 3,3′- diaminobenzidine—DAB—(Sigma) to specifically detect membrane-bound secondary antibody. The membrane was then washed with distilled water and dried at room temperature. All washes and incubations were conducted at room temperature with gentle shaking.

Hemagglutination assays

Hemagglutination assays were carried out in 1.5 ml eppendorfs. Lectin samples were prepared by doing series of 1:2 dilutions from a starting concentration of pure protein of 0.2 mg/ml. Lectin samples (100 μl) were incubated with 100 μl of rabbit erythrocytes (2% (v/v) in 0.15 M NaCl) at 37 °C for 30 min. The degree of agglutination was monitored visually after the tubes had been left at 37 °C for 30 min and subsequently left at room temperature for another 30 min. Samples that yielded no visible agglutination activity after these incubation steps were regarded as negative.

Fluorescence studies

Fluorescence measurements were performed at room temperature using a Varian Cary Eclipse Fluorescence Spectrophotometer. A quartz cuvette (10 mm path length) with 0.7 ml volume was used in the measurements. Excitation and emission band passes of 5 nm were used. Lectin solutions in PBS buffer were used at the concentration 0.08–0.1 mg/ml.

Fluorescence lectin spectra determination: Lectin samples were excited at 280 nm and the fluorescence emission was monitored in the 290–450 nm range.

Association constants determination: The association constants (Ka) for the binding of Me-α-galactose (Sigma) to native and recombinant frutalin were determined based on the changes in the lectin intrinsic fluorescence, as described in the literature

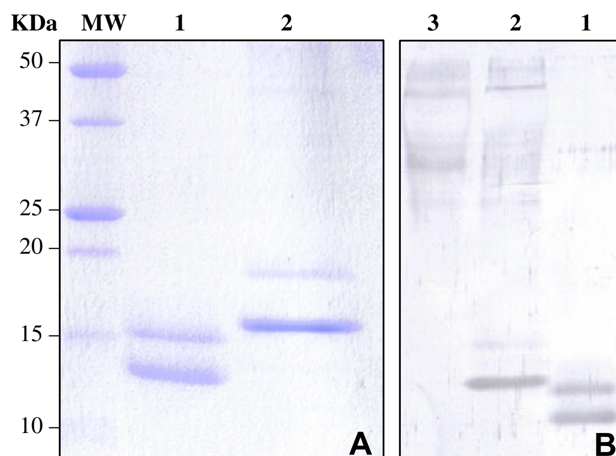

Fig. 2. SDS–PAGE (A) and Western blot (B) analysis of supernatants from a *P. pastoris* transformant expressing recombinant frutalin and of native frutalin isolated from *A. incisa* seeds. Legend: 1, native frutalin; 2, recombinant frutalin; 3, negative control; MW, molecular weight standards.

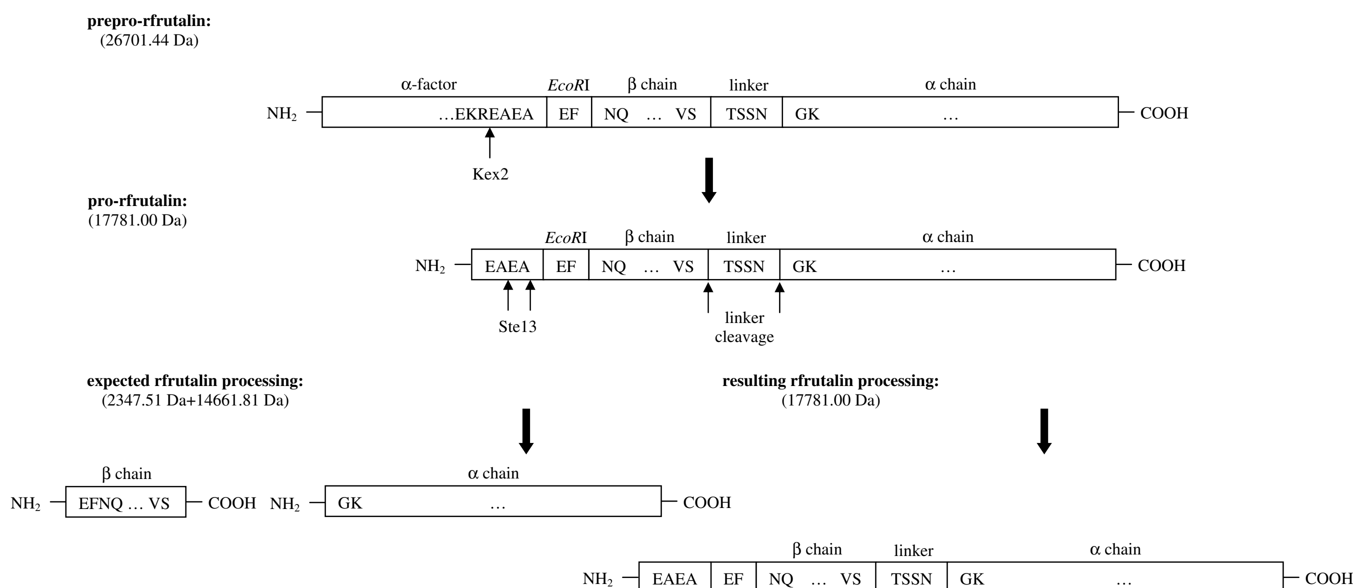

Fig. 1. Schematic representation of the processing of the recombinant frutalin construct expressed in the yeast *P. pastoris*. The processing of the signal sequence is made by the Kex2 protease, which cleaves between the arginine (R) and the glutamic acid (E) residues in the sequence "E-K-R-E-A-E-A", and by the Ste13 protease, that removes the "E-A-E-A" repeats. In the native frutalin processing, the four amino-acid linker peptide is excised, resulting in two polypeptide independent chains, α and β. The resulting recombinant frutalin contains six additional amino-acids ("E-A-E-A-E-F") at its N-terminus and the linker remains linked to the α and β chains. The calculated molecular mass for each stage of the recombinant frutalin processing is indicated on the figure.

[13,14]. The enhancement in the intrinsic fluorescence of native and recombinant frutalin was recorded at 333 and 335 nm, respectively, after addition of the non-fluorescent ligand Me-α-galactose until there was no significant change in the enhanced fluorescence (4 mM for native frutalin and 200 mM for recombinant frutalin). The sugars D-galactose, D-mannose, Me-α-mannose, D-glucose, Galα1-6Glu and Galβ(1-3)GalNac (all obtained from Sigma) were also added to both lectin samples in order to determine the maximum enhancement in the intrinsic fluorescence caused by each sugar.

Results and discussion

Frutalin gene synthesis/modification and transformation of P. pastoris

In order to prevent limitations in the expression of frutalin in *Pichia* due to codon bias utilisation, frutalin synthetic gene construction was employed as a cloning strategy to express frutalin in *P. pastoris*. Frutalin gene sequence encoding one of its mature isoforms was obtained using base synthesis and PCR approaches, as described in Materials and methods. A modified gene of frutalin α chain was artificially synthesised and the β chain plus the linker was successfully linked to the α chain by PCR using a 100 bp "Megaprimer". In DNA synthesis and primer design the codons that were rarely used in the protein expression system of *P. pastoris* were replaced with codons commonly used in this yeast. The sequence of the PCR product obtained, encoding complete frutalin gene sequence, was confirmed by sequencing and was consistent with the amino-acid frutalin sequence.

Optimised frutalin base sequence was placed under the control of the methanol-inducible *AOX*1 promoter, present in the pPICZαA expression vector, and transformed into the genome of *P. pastoris* KM71H yeast strain by electroporation. A schematic representation of the processing of the recombinant frutalin that it is expected to occur in *P. pastoris*, as well as the resulting recombinant frutalin, is shown in Fig. 1.

The insertion of the frutalin gene into the transformants was checked by "colony PCR" using frutalin-specific primers (data not shown). The ones that yielded a positive result in the PCR and showed high Zeocin resistance were randomly chosen to perform batch fermentations. Selected transformants were capable of growing with an antibiotic concentration 10 times higher than the one used in their initial selection (1 mg/ml).

Expression and purification of recombinant frutalin

The *Saccharomyces* α-factor preprosequence, included in the pPICZαA expression vector, was used as signal sequence for the secretion of the recombinant frutalin. Hence, supernatants from methanol-induced cultures were analysed for the presence of the recombinant frutalin by SDS–PAGE electrophoresis and Western blot analysis, after being treated as described in Materials and methods. As expected, recombinant frutalin was successfully expressed in *P. pastoris* as a secreted protein (Fig. 2A).

Recombinant frutalin could be observed in SDS–PAGE as a double band, both bands having a molecular weight higher than 15 kDa, as shown in Fig. 2A. Those bands were not detected in the fermentation supernatant of *P. pastoris* bearing only the empty pPICZαA expression vector (Fig. 2B, lane 3). Western blot analysis showed that the double recombinant frutalin band was recognised by native frutalin antibody as occurring with the double native frutalin band (Fig. 2B). Although recombinant and native frutalin have identical SDS–PAGE migration patterns, recombinant frutalin bands have higher molecular weight than native frutalin bands, as can be observed in Fig. 2. Further characterisation of the two recombinant frutalin bands was conducted.

Recombinant frutalin was purified from methanol-induced culture supernatants by size-exclusion chromatography using a Sephacryl® S-100 HR column. In Fig. 3 is shown a typical chromatography profile of the recombinant frutalin purification from a 50 ml methanol-induced culture. Pooled fractions resulted in a total amount of about 18–20 mg/l of pure recombinant frutalin.

Sugar binding studies

The fluorescence emission spectrum of the purified recombinant frutalin showed an emission maximum at 335 nm. Native frutalin presents a fluorescence emission maximum at 333 nm [7].

No significant change in the fluorescence emission was detected when, D-galactose, D-mannose, Me-α-mannose, D-glucose, mellibiose (Galα1-6Glu) and Galβ(1-3)GalNac (T-antigen) were added to the recombinant frutalin at a final concentration of 4 mM. However, after addition of 4 mM Me-α-galactose to the recombinant lectin, an enhancement of about 6% in the recombinant frutalin fluorescence was observed. When the above-mentioned sugars were added to the recombinant frutalin at higher concentrations (100 mM), a 5% enhancement in the fluorescence emission could also be observed in the case of mellibiose. On the other hand, when D-galactose, Me-α-galactose, mellibiose or Galβ(1-3)GalNac was added to the native frutalin very high enhancements in the fluorescence emission were detected (for instance, 35% for Me-α-galactose) and little or no change was observed for the other sugars (all 4 mM). These data clearly show that the recombinant frutalin carbohydrate specificity is qualitatively very similar to the carbohydrate specificity of the native frutalin, nevertheless the former presents less binding affinity. This is also corroborated by the recombinant

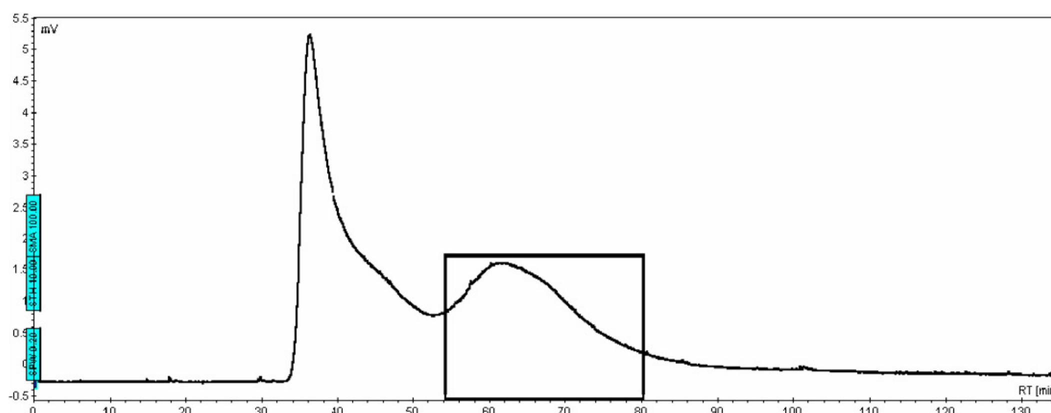

Fig. 3. Elution profile on a Sephacryl® S-100 HR column of the supernatant obtained from a 50 ml methanol-induced culture. The boxed period time corresponds to the fractions containing pure recombinant frutalin.

and native lectin association constants with the monosaccharide Me-α-galactose (Table 1).

Molecular and biological characterisation of the recombinant frutalin

Galactose-specific jacalin-related lectins are synthesised as preprolectins, consisting of a signal sequence, a pro-peptide, a β chain, a linker tetrapeptide, and an α chain. An evolutionarily event in the Moracea family, the inclusion of a signal sequence and vacuolar targeting sequences in mannose-specific lectins could have resulted in galactose-specific lectins [15]. In mature lectin, the signal sequence and the pro-peptide are moved through post- and/or co-translational processing, and the linker is also excised to generate two independent polypeptide chains, α and β. The plant mechanism that is behind lectin linker cleavage is not yet clear. The linker amino-acid composition seems to be well conserved in several galactose-binding jacalin-related lectins. Jacalin [16], frutalin (as shown in this work) and morniga G (*Morus nigra* galactose-specific jacalin-related lectin) [17] present the same amino-acid linker sequence (T-S-S-N). Its role in plant preproprotein (before lectin processing) is still unclear: one hypothesis is that it may consist of a temporal lectin inactivation to prevent lectin interaction with glycoproteins in the endoplasmic reticulum and/or Golgi compartment [15].

Table 1
Association constants (Ka) for the binding of the monosaccharide Me-α-galactose to native and recombinant frutalin based on the changes in the intrinsic fluorescence emission

Lectin	Me-α-galactose-binding constants (Ka, M^{-1})
Native frutalin	1.56×10^4 ($\pm 0.71 \times 10^3$)
Recombinant frutalin	1.38×10^2 ($\pm 0.65 \times 10^1$)

The data are an average of two independent measurements with the respective standard deviations.

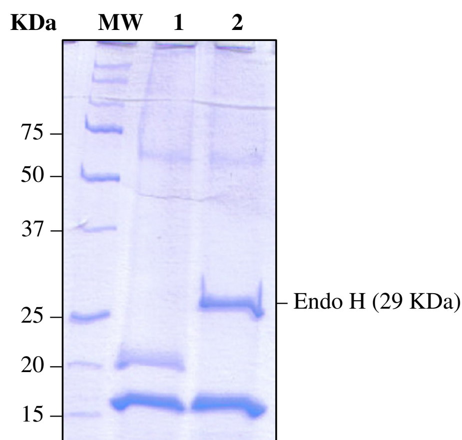

Fig. 4. SDS–PAGE analysis of recombinant frutalin treated with Endoglycosidase H. Legend: lane 1, recombinant frutalin before Endoglycosidase H treatment; lane 2, recombinant frutalin after Endoglycosidase H treatment; MW, molecular weight standards.

Native frutalin migrates in SDS–PAGE as a double band, as a result of the processing of the "preprolectin" (Fig. 2A). The upper band corresponds to the α chain of the glycosylated isoforms (highly glycosylated), whereas the lower band represents the α chain of the non-glycosylated isoforms (or slightly glycosylated). The β chain is not visible due to its low molecular weight (2.1 kDa). The molecular weight difference between recombinant and native frutalin lower bands on SDS–PAGE indicated that the frutalin linker was not excised (Fig. 2A). Furthermore, the molecular weight of recombinant frutalin lower band on SDS–PAGE has a good agreement with the calculated molecular weight based on the amino-acid residues of β and α chains connected through the linker (17.1 kDa). The amino-terminal sequencing reaction of purified recombinant frutalin, determined by Edman degradation, confirmed that the linker was not excised and that recombinant frutalin was unequivocally expressed as a single chain protein, *i.e.* the β chain C-terminus remains linked to the α chain N-terminus by the four linker amino-acid peptide (Fig. 1). The amino-terminal sequencing reaction also showed that the α-factor prosequence was not correctly processed. The amino-acids "E-A-E-A" were detected at the N-terminal of the recombinant frutalin, which correspond to the double Glu-Ala amino-acids of the α-factor C-terminal (Fig. 1). This result indicates that the cleavage by *STE13* gene product (Ste13 aminopeptidase), which removes Glu-Ala repeats, was inefficient. Incorrect processing of the α-factor is commonly observed in the expression of heterologous proteins in *P. pastoris* using this signal sequence [18,19]. On the other hand, N-terminal sequencing results showed that β chain and the N-terminal α chain amino-acids were in complete agreement with the cloned nucleotide sequence (Fig. 1).

The generation of a free glycine amino group residue, localised at the N-terminus of α chain, by the correct excision of the linker peptide seems to be essential for the formation of an active galactose-binding site [4]. However, recombinant frutalin, expressed as a single chain protein, *i.e.* without proteolytic linker cleavage, showed ability to bind Me-α-galactose. This work corroborates the hypothesis that suggests that jacalin-related lectins specificity is not entirely dependent on the generation of a free glycine amino-

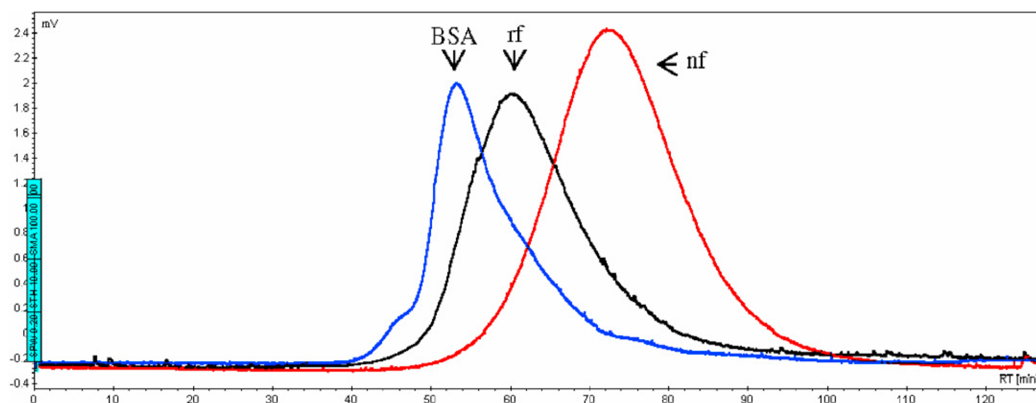

Fig. 5. Size-exclusion chromatogram of BSA, recombinant frutalin (rf) and native frutalin (nF) eluted on a Sephacryl® S-100 HR column (protein concentrations: BSA-1 mg/ml; native frutalin-1.2 mg/ml; recombinant frutalin-0.9 mg/ml).

acid at the N-terminus of the α chain generated by linker excision, although this processing step may be required to obtain higher affinity [20,21]. Further studies on recombinant frutalin binding site and structure have to be conducted to elucidate its binding specificity.

The higher molecular weight band of recombinant frutalin in SDS–PAGE (upper band in lane 1 of Fig. 4) may correspond to a partial glycosylation, since the cloned α chain has one potential site for N-glycosylation (Asn74). After recombinant frutalin treatment with Endoglycosidase H, the recombinant frutalin upper band was resolved from the SDS–PAGE gel (Fig. 4, lane 2), confirming that the recombinant frutalin is partially N-glycosylated.

Native frutalin exhibited a strong hemagglutinating activity towards rabbit erythrocytes, as reported earlier [5–7]. However, no visible hemagglutinating activity was detected with purified recombinant frutalin in the concentration range 0.002–0.2 mg/ml. Hemagglutination activity presupposes at least two binding sites (by dimer formation); otherwise it cannot be detected. The *P. pastoris* glycosylation pattern did not inhibit frutalin native structure (tetramer), since recombinant frutalin eluted from a Sephacryl® S-100 HR column between BSA (66 kDa) and native frutalin (48–49 kDa) [5], with an estimated molecular mass of approximately 62–64 kDa (Fig. 5). However, it is possible that the *P. pastoris* glycosylation pattern inhibits the hemagglutination activity, since the presence of the linker does not seem to inhibit the hemagglutinating activity in the related lectin–jacalin. Sahasrabuddhe et al. [20] have cloned jacalin in *E. coli* and a recombinant non-glycosylated single chain lectin with hemagglutinating activity was obtained. Both reports on recombinant lectins produced in *P. pastoris* with hemagglutinating activity [18–19,22–26] and without hemagglutinating activity [27] can be found in the literature.

Conclusion

For the first time a galactose-binding lectin was expressed and purified from the methylotrophic yeast *P. pastoris*. Although linker cleavage between α and β chains has not occurred in the *P. pastoris* protein expression system, characterisation of the recombinant lectin revealed a carbohydrate-binding specificity similar to that reported for the native frutalin lectin. After purification by size-exclusion chromatography, 18–20 mg/l of recombinant frutalin was typically obtained . Thus, frutalin can be easily produced and purified in the heterologous *P. pastoris* system, which is known to be an excellent host for large-scale production. In future experiments optimisation of frutalin expression and large-scale-up for production and purification will be conducted.

Acknowledgments

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