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Effects of NaCl and pH on the structural conformations of kidney bean vicilin

Sule Mundi, Rotimi E. Aluko *

Department of Human Nutritional Sciences, University of Manitoba, Winnipeg, Manitoba, Canada R3T 2N2
The Richardson Centre for Functional Foods and Nutraceuticals, University of Manitoba, Winnipeg, Manitoba, Canada R3T 2N2

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ABSTRACT

Structural changes as a result of variations in pH value and salt concentration were determined for purified vicilin, the major globular protein in kidney beans using intrinsic fluorescence and circular dichroism (CD). The vicilin consisted of two polypeptide chains of about 43 and 45 kDa in size when analysed under reducing SDS–PAGE. Amino acid analysis showed that the vicilin had high contents of acidic amino acids and a low lysine/arginine ratio. Intrinsic fluorescence measurements were performed to measure exposure of tyrosine and tryptophan as a means of estimating protein conformational changes. Generally, the vicilin showed an unfolded structure at pH 3.0, 5.0, 7.0, and 9.0 as evident by the extensive red shift (>350 nm) of the wavelength of maximum tryptophan fluorescence intensity. At pH 3.0 and 5.0, the fluorescence intensity (FI) was greater than values obtained at pH 7.0 and 9.0, which suggests that the microenvironment of tryptophan was less hydrophilic at acidic pH. Addition of NaCl also led to increased FI, an indication of structural changes of tryptophan in response to the hydrophilic environment. These changes in FI were due mostly to tryptophan emission because tyrosine emission (at 303 nm) was suppressed. The far-UV CD spectra showed that vicilin had minimal measurable secondary structures at pH 3.0 and 5.0 when compared to pH 7.0 and 9.0. Addition of NaCl led to an increase in the tertiary structure conformation of vicilin as determined from the near-UV CD spectra.

1. Introduction

Pulses, including kidney beans (*Phaseolus vulgaris* L.), are high in dietary fibre and low in fat, making beans a good source of cholesterol and blood sugar lowering food, which can reduce the risk of cardiovascular diseases and type 2 diabetes (Aykroyd & Doughty, 1982). In addition, they are high in proteins (20–30% on a dry weight basis) (Yin, Tang, Wen, & Yang, 2010a), which put them among some of the richest food sources of proteins for human and animal nutrition, as well as for food industry applications. The main storage protein in kidney beans is the globular phaseolin, also known as vicilin (Yin et al., 2010a). Depending on the cultivar, vicilins are composed of two (dimer) or three (trimer) polypeptide chains (Romero, Sun, McLeester, Bliss, & Hall, 1975). In addition to phaseolin, kidney beans also contain lectins, which are glycoproteins that reversibly and non-enzymatically bind to specific sugars, and in doing so, play a range of crucial roles in many cell–cell recognition events that trigger several important cellular processes (Chandra et al., 2006). All of the biological functions reported for proteins are directly affected by the structure and other physical properties such as size, charge and hydrophobicity of the protein, which affect interactions with or affinity for ligands (Chandra et al., 2006). Environmental factors can cause conformational changes and specific alterations in the function of the protein. For example, the presence of protein structure perturbants (e.g. sodium dodecyl sulfate, urea and dithiothreitol) and other environmental parameters, such as pH, ionic strength, binding of ligands and temperature can affect molecular organization, which influences biological functions. Thus, understanding the impact of protein conformation under various buffer conditions on the biological functions remains a primary focus in protein chemistry, especially during various food processing events. Cooking and other forms of food preparation or processing are usually necessary for the reduction of anti-nutrient factors in kidney beans (Shimelis & Rakshit, 2007). During processing events, the conditions in the food environment changes and hence, the structure could be affected. The knowledge of changes in temperature, pH, or ionic strength, and their effects on structure–function relationships as well as their control during food preparation is essential.

Spectroscopic methods, including circular dichroism (CD) and fluorescence spectroscopy (FS) of proteins can provide sensitive indications of the conformational changes that may occur (at the molecular level) due to changes in the protein environment. CD and FS of proteins provide important structural information concerning the details of the helical content of proteins or the asymmetric environment of aromatic residues. Previous studies (Dyer,

* Corresponding author. Tel.: +1 204 474 9555; fax: +1 204 474 7593.
E-mail address: rotimi.aluko@ad.umanitoba.ca (R.E. Aluko).

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Nelson, & Murai, 1992; Tang and Ma, 2009; Tang, Sun, & Foegeding, 2011; Yin, Tang, Wen, Yang, & Yuan, 2010b; Yin, Tang, Yang, & Wen, 2011b; Yin et al., 2010a, 2011a) have investigated the effects of protein structure perturbants and pH on the conformational properties of phaseolin and kidney bean isolate (KPI). By using CD, FS and differential scanning calorimetry (DSC), protein conformational and structural changes due to heat (Dyer et al., 1992; Tang and Ma, 2009), succinylation and acetylation (Yin et al., 2010b) and glycation (Tang et al., 2011) have been determined. The effects of polar, neutral, anionic and cationic quenchers such as acrylamide, nitrate and caesium ion respectively have also been studied (Yin, Tang, Yang, & Wen, 2011b). The results showed significant changes in the α-helix content and highly ordered secondary structures (α-helix + β-strand) in addition to tertiary conformation unfolding and subsequent rearrangement process. Yin et al. (2011a) studied the effects of pH on the secondary and tertiary conformations of phaseolin by using CD spectra in near/far UV region and showed that there were changes in protein conformation with shifts in pH values. However, there is paucity of information on the combined effects of varying NaCl concentrations and pH on the CD and FS properties of purified kidney bean globular protein (vicilin). Therefore, the goal of the current research was to study the effects of changes in pH alone or in combination with NaCl concentrations on the structural conformations of purified kidney bean vicilin using CD and FS.

2. Materials and methods

2.1. Preparation of crude globulin sample

Red kidney bean seeds were obtained from a local store in Winnipeg and were ground into flour using a Retsch ZM200 centrifuge mill (Retsch GmbH, Haan, Germany). Globulin proteins were extracted from the flour according to the previously described ammonium sulfate precipitation method (Aluko, 2004). The major globulin fraction in kidney beans was obtained by adjusting an aqueous extract (obtained using 0.1 M phosphate buffer, pH 7.0 containing 0.4 M NaCl) of the flour to 40% ammonium sulfate saturation, in order to precipitate albumins and other smaller proteins and enzymes. After centrifugation (9000*g*, 45 min, 4 °C), the supernatant was then brought to 80% ammonium sulfate saturation to precipitate the globulins. The ammonium sulfate was then removed from the isolated globulins by dialyzing sample against water at 2 °C using dialysis membrane with molecular weight cut-off of 6–8 kDa. The dialysis bag content was centrifuged (9000*g*, 45 min, 4 °C) and the resultant precipitate (globulin) was freeze-dried and further purified using Fast Protein Liquid Chromatography (FPLC).

2.2. Purification of vicilin by FPLC

The kidney beans globulin protein was separated using ÄKTAPurifier FPLC system equipped with a size exclusion column, HiLoad 26/60 Superdex 200 Prep grade (GE Healthcare, Montreal, PQ). Sodium phosphate (0.1 M) prepared to contain 0.4 M NaCl was used as the equilibration buffer for the column, and as an elution buffer. Two millilitres of 100 mg/mL globulin protein solution, filtered through 0.2 μm filter disks were loaded onto the Superdex column and run at a flow rate of 2.5 mL/min; eluted proteins were detected from the UV absorbance at 214 nm. The major fraction was collected and desalted using Hiprep 26/10 desalting column, following which the sample was freeze dried, analysed for protein content (Markwell, Haas, Bieber, & Tolbert, 1978) and stored at −20 °C until further analysis.

2.3. Sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS–PAGE)

The freeze dried globulin fraction was subjected to SDS–PAGE according to the method of Aluko and McIntosh (2004) with minor modifications. Ten milligramme protein was dispersed in a Tris/HCl buffer (pH 8.0) containing SDS and mercaptoethanol, each at 10% (w/v), followed by heating at 95 °C for 10 min, cooling and centrifugation (15 000*g*) for 5 min. After centrifugation, approximately 1 μL of supernatant was loaded onto an 8–25% gradient gel and electrophoresis performed with a Phastsystem Separation and Development unit according to the manufacturer's instructions (GE Healthcare, Montréal, PQ). A mixture of Promega standard proteins (10–225 kDa) was used as the molecular weight marker (Fisher Scientific, Oakville, ON, Canada).

2.4. Amino acid analysis

HPLC system was used for the analysis of the amino acid profiles after samples were hydrolyzed with 6 M HCl according to the method described by Bidlingmeyer, Cohen, and Tarvin (1984). The cysteine and methionine contents were determined after performic acid oxidation (Gehrke, Wall, & Absheer, 1985) and tryptophan content was determined after alkaline hydrolysis (Landry & Delhaye, 1992).

2.5. Intrinsic fluorescence emission spectrophotometry

Intrinsic fluorescence spectra were recorded on the JASCO FP-6300 spectrofluorimeter at 25 °C using a 1 cm pathlength cuvette. Protein stock solution was diluted to 0.002% (w/v) with respective buffers or buffers that contain NaCl. Fluorescence spectra were recorded at excitation wavelengths of 275 (tyrosine + tryptophan) and 295 (tryptophan) with emission recorded from 280 and 300 to 500 nm, respectively, using 2.5 nm band width (Schmid, 1989). Emissions of the buffer or buffer + NaCl blanks were subtracted from those of the respective samples to obtain fluorescence spectra of the sample.

2.6. Measurements of circular dichroism (CD) spectra

CD measurements were carried out at 25 °C in a J-810 spectropolarimeter (JASCO, Tokyo, Japan) using the spectral range of 190–240 nm (far UV) for secondary structure determinations and 250–320 nm (near UV) for tertiary structure (Schmid, 1989). The vicillin was solubilized in 10 mM phosphate buffer (pH 3.0, 5.0, 7.0, and 9.0) that contained appropriate NaCl concentration followed by centrifugation (10 000*g*) for 30 min (to remove particulate matter) and supernatant used for CD structural analysis. For secondary structure determinations, a cuvette with pathlength of 0.05 cm was used and contained 1 mg/ml protein solution while the tertiary structure was measured in a 0.1 cm cuvette that contained 2 mg/ml protein concentration. Vicilin at 1 and 2 mg/ml (0.1% and 0.2% w/v, respectively) was completely soluble at all the pH values used; solubility range of vicilin is 5% at pH 5.0 and 75% at pH 9.0. All the CD spectra were obtained as the average of three consecutive scans with automatic subtraction of the buffer or buffer + NaCl spectra.

3. Results and discussion

3.1. Fast protein liquid chromatography (FPLC) purification and gel electrophoresis

Kidney beans globulin proteins were separated based on the differential exclusion from the pores of the column's packing