Journal of Cereal Science 66 (2015) 53–58

Contents lists available at ScienceDirect

Journal of Cereal Science

journal homepage: www.elsevier.com/locate/jcs

Fluorimetric studies of the interactions of wheat puroindolines with polar lipids on the surface starch granules

Justyna Rosicka-Kaczmarek [a, *], Maria Stasiuk [b], Ewa Nebesny [a], Aleksandra Komisarczyk [a]

[a] Lodz University of Technology, Faculty of Biotechnology and Food Sciences, Institute of Chemical Technology of Food, Stefanowskiego 4/10, 90-924 Lodz, Poland

[b] University of Wroclaw, Faculty of Biotechnology, Department of Lipids and Liposomes, Joliot-Curie 14a, 50-383 Wroclaw, Poland

ARTICLE INFO

Article history:
Received 23 June 2015
Received in revised form
7 October 2015
Accepted 28 October 2015
Available online 2 November 2015

Keywords:
Puroindolines–lipid interaction
Polar lipids
Intrinsic fluorescence
fluorescence polarization

ABSTRACT

The interactions of puroindolines with polar lipids were investigated using polarization of fluorescence probes preincorporated into a liposomal bilayer containing PC, PI, PS, MGDG, DGDG, and sulfolipids. The intrinsic fluorescence of Trp residue method was also used. Regardless of the kind of lipid used for liposome preparation, proteins interacted with the liposomes. Conformational changes of the proteins were observed simultaneously with the change in the molecule packing in the lipid bilayer of the liposomes. Puroindoline interactions with the surface of the liposomes have explicit importance for the net charge of this surface. The strong interaction between the proteins and lipids takes place in the presence of a ligand with a negative charge. The obtained results confirm that lipids take part in puroindoline–starch granule surface interactions.

1. Introduction

Proteins and lipids are the most important groups of plant compounds from a technological point of view. The quantities of these compounds, which are bound to starch, depend on the origin of the plant, the amount of starch, and the degree of starch purification during its isolation from a raw material (Baldwin, 2001; Debet and Gidley, 2006; Finnie et al., 2010). A typical, well-purified plant-derived starch sample has an average 0.25% protein content and 1% lipid content.

Of all the protein types located on the surface of the wheat starch granule, a group of friabilins/puroindolines with a molar mass of about 15 kDa is noteworthy due to their properties (Darlington et al., 2000; Douliez et al., 2000). It is suggested that puroindolines are the most important genetic elements (biomarkers) of the wheat grain, and that they can control its hardness (Hogg et al., 2004). They are an important factor in efforts to increase the yield and quality of cereals (Marion et al., 2007).

Two main isoforms can be distinguished in the group of puroindolines – PIN-a and -b. These two groups differ in the amount of tryptophan residues present in their structures (Turnbull et al., 2003; Day et al., 2006; Feiz et al., 2009a). The major isoform, PIN-a, contains a unique tryptophan-rich domain (Trp–Arg–Trp–Trp–Lys–Trp–Trp–Lys) (Blochet et al., 1993). This domain has a different amino acid sequence (Trp–Pro–Thr–Trp–Trp–Lys) in PIN-b, which is a minor isoform found in most wheat cultivars (Gautier et al., 1994). The unique and diverse biological and technological functions of puroindolines are closely related with their structure and ability to bind lipids, which is influenced by a change in the structure and topology of hydrophobic chamber (tryptophan-rich domain) (Marion et al., 2007; Feiz et al., 2009b).

Studies conducted by Greenblatt et al. (1995) indicate that polar lipids (glycolipids and phospholipids) play an important role in the interactions of puroindolins with the starch surface. According to Oda and Schofield (1997), lipid participation in this process might be indirect, with lipids acting as a bridge between starch and proteins, or direct, with lipids possibly causing conformational changes in proteins, allowing them to bind with starch. Precise mechanisms of those interactions have not been fully established yet, but it is believed that the tryptophan domain plays a significant role in it. Some aspects of these interactions remain unclear, such as the

Abbreviations: LUV, large unilamellar vesicles; NBD-PE, N-(7-nitro-2-1,3-benzoxadiazol-4-yl) dioleoylphosphatidylethanolamine; TMA-DPH, 1-[4-(trimethylammonium) phenyl]-6-phenyl-1,3,5-hexatriene; PS, phosphatidylserine; PC, phosphatidylcholine; PI, phosphatidylinositol; PE, phosphatidylethanolamine; SA, stearylamine; SQH, sulfolipid; MGDG, monogalactosyldiglyceride; DGDG, digalactosyldiglyceride; PIN, puroindoline protein; PIN-a, puroindoline-a protein; PIN-b, puroindoline-b protein; Trp, tryptophan.

* Corresponding author.
E-mail address: justyna.rosicka-kaczmarek@p.lodz.pl (J. Rosicka-Kaczmarek).

http://dx.doi.org/10.1016/j.jcs.2015.10.011
0733-5210/

protein affinity to various types of lipids; the influence of electrostatics on lipid-PIN interactions; the role of the protein tryptophan domain in this process; and finally the influence of lipid binding on PIN conformation (Clifton et al., 2007, ; Wanjugi et al., 2007; Dubreil et al., 1997).

Fluorescence spectroscopy has long served as a sensitive and versatile tool for measuring membrane–protein interactions. It presents the advantages of extreme sensitivity and minimal perturbation, and it can provide a wealth of information on membrane–protein interactions, such as the affinity of the peptide for the membrane phase, its location and orientation in the bilayer, its aggregation state, and its effects on membrane structure and integrity. When incorporated into the protein molecules and/or located in the phospholipid bilayer, fluorescent dyes allow the observation of protein–lipid interactions in terms of changes in the conformation of the protein and changes in the level of membrane phospholipid molecule packing.

In this study, we investigated the interaction of puroindolines and the liposomal bilayer by measuring the effect of puroindolines on the structural order of lipid membranes (using the steady-state fluorescence polarization of TMA-DPH and NBD-PE), the membrane electrostatic surface potential (using fluorescein-PE), and protein conformation changes (using the intrinsic tryptophan residue fluorescence). In addition, we also examined changes in the intrinsic fluorescence of Trp residues under the interaction of protein with a methanolic solution of PC, polar lipids extracted from the starch granule surface, glucose, maltose, and arabinoxylans.

2. Materials and methods

Puroindolines were generously provided by Didier Marion and his co-workers from Unité de Biochimie et Technologie des Protéines, I.N.R.A. (Nantes, France). All of the phospholipids were purchased from Lipid Products (Nutfield, Surrey, Great Britain). All of the fluorescent probes were from Molecular Probes (Eugene, OR, USA). The remaining chemicals were of the best available purity from CHEMPUR (Piekary Slaskie, Poland). Glucose and maltose were purchased from Sigma–Aldrich.

2.1. Methods

2.1.1. Preparation of liposomes

Large unilamellar vesicles (LUV) were prepared from egg PC, PC:PS, PC:MGDG, PC:DGDG, PC:SA, PC:PI, or PC:SQH in the molar ratios 95:5 and 80:20. Lipids, with or without the fluorescent probes fluorescein-PE, NBD-PE or TMA-DPH (when used, these were at the final concentration in the liposomal membrane of 1 mol % versus total lipid concentration), were dissolved in chloroform or chloroform:methanol 1:1 (by volume) and mixed in the appropriate ratios. The chloroform was removed under a stream of nitrogen, and the lipid residue was subsequently maintained under a reduced pressure for approximately 1 h. The dry lipids were hydrated at room temperature in 10 mM Tris–HCl (pH 6.0). The resulting dispersions of multilamellar vesicles were extruded for ten times through a polycarbonate filter (100 nm pore-size, Nucleopore, Whatman Ltd) to obtain large unilamellar vesicles (Zhao et al., 2006).

2.1.2. The effect of puroindolines on lipid mobility and liposomal surface charge

The steady-state TMA-DPH or NBD-PE fluorescence polarization measurements (Excitation wavelength 355 nm, Emission wavelength 430 nm and Excitation wavelength 463 nm, Emission wavelength 536 nm, respectively) were obtained using a Varian Cary Eclipse spectrofluorometer (Varian, Mulgrave, Victoria, Australia) equipped with a thermostatic cuvette holder at 21 °C. A solution of puroindolines in 10 mM Tris–HCl (pH 6.0) was added to the appropriate liposome suspensions containing 0.125 mg of lipid mixtures in microliter amounts. The final amount of puroindolines in the incubation medium varied between 5 and 60 μg. The samples were incubated for 10 min after the addition of each portion of the protein and the value of fluorescence polarization was determined. Polarization values were calculated according to the standard procedure (Lakowicz, 1983).

The effect of the investigated protein on the membrane electrostatic surface potential was studied using the pH indicator fluorescein-PE. The prepared liposomes (0.125 mg per sample) were incubated for 10 min with puroindolines (from 5 to 60 μg per sample) at room temperature with continuous stirring, and the intensity of fluorescence was measured (Excitation wavelength 496 nm, Emission wavelength 519 nm). Other experimental conditions were as describe above.

2.1.3. The effect of liposomes on the intrinsic fluorescence of Trp residues of puroindolines

We monitored alterations in the microenvironment of the Trps of the peptide upon binding to the lipid bilayers by measuring its Trp fluorescence, as described previously (Zhao et al., 2006). The obtained liposomes were added to a solution of puroindolines (40 μg) of 10 mM Tris–HCl (pH 6.0), with continuous stirring in a total volume of 2 mL and maintained at 24 °C. Experiments were provided also with separately added methanolic solution of PC, the lipids extracted from the starch, or water solution/suspension of glucose, maltose, starch, and arabinoxylans. After 10 min of equilibration, Trp fluorescence spectra were measured with a Varian Cary Eclipse spectrofluorometer. The tryptophan residues of the puroindolines were excited at 295 nm and emission spectra were recorded from 300 to 400 nm. The spectra were corrected for the contribution of light scattering in the presence of vesicles or ligands.

2.1.4. Extraction of polar lipids from starch granule surface

Wheat starch surface lipids from the wheat variety *Elipsa* (soft) were extracted with propan-2-ol solution with water (90:10) using the method of De Oliveira and Finnie (De Oliveira, 2010; Finnie, 2009).

2.1.5. Isolation of arabinoxylans from wheat bran

The isolation of arabinoxylans from wheat bran was done using the method of Sun et al. (Sun et al., 2001).

2.1.6. Statistical analysis

All the experiments were performed in triplicate and experimental data were analyzed using Analysis of Variance (ANOVA), and expressed as mean value ± standard deviation. All statistical computations and analyses were conducted using SPSS version 13.0 for Windows.

3. Results and discussion

The effect of the studied proteins on the properties of the bilayer across its thickness was monitored by assessing alterations in the fluorescence polarization of probes localized at various depths of the bilayer. N-(-nitrobenz-2-oxa-1,3-diazol-4-yl)-1,2-dihexadecanoyl-sn-glycero-3-phosphoetanolamine triethylammonium salt (NBD-PE) was used in the hydrophilic region and 1-(4-trimethyl ammoniumphenyl)-6-phenyl-1,3,5-hexatrien p-toluenesulfonate (TMA-DPH) was used in the deeper region, 10.9 Å from the center of the membrane (Kaiser and London, 1998). It is worth noting that