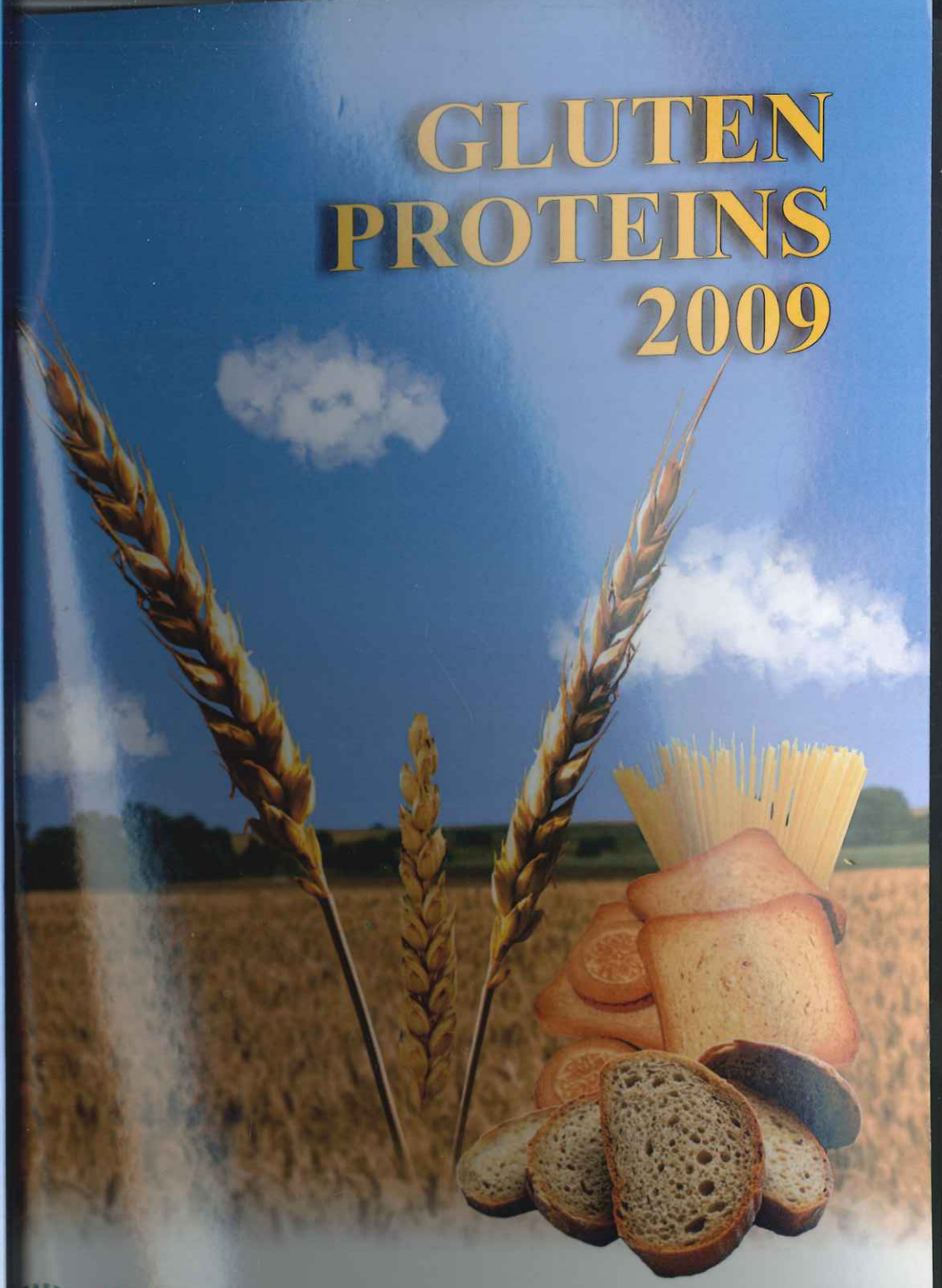
Interactions of gliadins with model membranes in confined geometry

A. Banc, L. Navailles, B. Desbat, Yves Popineau, Cecile C. Mangavel, Denis Renard

9 782738 012814

GLUTEN PROTEINS 2009



Coordinator : Gérard Branlard

Edited by : INRA, 147, rue de l'université 75 338 Paris Cedex 07 France

pLMWlmwBmyc **X** pHMWAx1FLAG
pLMWgliHA **X** pLMWlmwBmyc
pHMWAx1FLAG **X** pLMWgliHA

pLMWAx1FLAG **X** pHMWlmwBmyc
pHMWlmwBmyc **X** pHMWgliHA
pLMWAx1FLAG **X** pHMWgliHA

Fig 3: Transgenic line crossing combinations

CONCLUSIONS

Expression of epitope tagged gluten proteins in wheat is a novel tool to follow their patterns of trafficking and deposition.

Acknowledgement: Rothamsted Research receives grant-aided support from the Biotechnology and Biological Sciences Research Council (BBSRC) of the UK. Work described here was supported by BBSRC grant BB/C51271/0/1 The Relationship between Patterns of Gluten Protein Synthesis and Deposition and Grain Processing Quality: a Transgenic Approach

REFERENCES

Jones HD, Freeman JA and Sparks CA 2008. A Transgenic approach to understanding gene expression in cereals. In: Cereal Science and Technology for Feeding Ten Billion People: Genomics Era and Beyond. (Options méditerranéennes série A. Séminaires Méditerranéens, N° 81) Eds.: Molina Cano J.L., Christou P., Graner A., Hammer K., Jouve N., Keller B., Lasa J.M., Powell W., Royo C., Shewry P., Stanca A.M. EUCARPIA/CIHEAM. ISBN: 2-85352-404-3.

Shewry, P. R., Tosi, P., Jones, H. D., Sparks, C., Gritsch, C., Funatsuki, W., Niwa, K., Huttly, A., Napier, J. A., D'Ovidio, R. & Freeman, J. 2007. Using epitope tagging to explore the trafficking, location and functional properties of wheat gluten proteins. In *Gluten ptroteins 2006* G. L. Lookhart & P. W. K. Ng, eds, 112-116. AACC International, St Paul

Stöger, E, Parker, M., Christou, P., and Casey, R. 2001. Pea legumin overexpressed in wheat endosperm assembles into an ordered paracrystalline matrix. Plant Physio., 125: 1732-1742.

Toole, G.A., Wilson, R.H., Parker, M.L., Wellner, N.K.,Wheeler, T.R., Shewry, P.R. and Mills E.N.C. 2007. Remodelling of arabinoxylan in wheat (Triticum aestivum) endosperm cell walls during grain filling. Planta, 229:667-80.

Tosi, P., Parker, M., Sanchis Gritsch, C., Carzaniga, R., Martin, B. and Shewry, P.R. 2009. Trafficking of storage proteins in developing grain of wheat J. Exp. Bot., 60: 979-991.

Tosi, P., Masci, S., Giovangrossi, A., D'Ovidio, R., Bekes, F., Larroque, O., Napier, J., and Shewry, P.R. 2005. Modification of the low molecular weight (LMW) glutenin composition of transgenic durum wheat:effects on glutenin polymer size and gluten functionality. Mol. Breeding, 16:113-126.

Interactions of gliadins with model membranes in confined geometry: a physicochemical approach for the initiation of protein bodies in the ER lumen

A BANC [1†], L. NAVAILLES [1], B. DESBAT [2], Y. POPINEAU [3], C. MANGAVEL [3] and D. RENARD [3]
[1]Université Bordeaux-1 CNRS, Centre de Recherche Paul-Pascal, F-33600 Pessac, France.
[2]Université Bordeaux-1, Laboratoire de Chimie et Biologie des Membranes et Nanoobjets, UMR 5248-CNRS, ENITAB, F-33607 Pessac, France.
[3]UR1268 Biopolymères Interactions Assemblages, INRA, F-44300 Nantes, France
†Current address: CEA/CNRS/Université Montpellier II, UMR 5257, Institut de Chimie Séparative de Marcoule, F-30207 Bagnols sur Cèze, France

ABSTRACT

The mechanisms leading to the assembly of wheat storage proteins in protein bodies within the endoplasmic reticulum (ER) of endosperm cells are still not well understood. In this work, physical chemistry parameters which could be involved in these processes were explored. The dynamic behavior of γ-gliadins inserted in lyotropic lamellar phases was studied using FRAP in order to model the confined environment of proteins within the ER. Changes in the diffusion coefficient as a function of lamellar periodicity enabled us to propose an interaction between γ-gliadins and membranes. The interaction was studied in more detail with the help of phospholipids (Langmuir monolayers). γ- and ω-gliadins were injected under DMPC and DMPG monolayers and the 2D systems were studied by Brewter Angle Microscopy (BAM), and surface tension measurements. Results showed that both gliadins adsorbed under monolayers and formed micrometer-sized domains at equilibrium. However, when probed by reflectance measurements, ω-gliadin aggregates displayed a constant thickness consistent with a monolayer, while the thickness of γ-gliadin aggregates increased with the quantity of protein injected. All these findings enabled us to propose a model for the self-assembly of gliadins via a membrane interface and to highlight the predominant role of the wheat prolamin repetitive domain in the membrane interaction. In the biological context, these results suggest that the repetitive domain could be an anchor for the interaction with the ER membrane and a nucleus point for the formation and growth of protein bodies.

INTRODUCTION

Wheat storage proteins (WSP) are very important for a range of technological applications from baking performance,[1] to the elaboration of biomaterials,[2] and the development of new drug delivery systems.[3] WSP display a very extensive polymorphism, but they present a common feature which is the presence of repetitive sequences in their primary structures. Prolamins are subdivided into monomeric gliadins and polymeric glutenins. In the biological context, WSP serve as a source of amino acids for germination, and are mainly stored within protein bodies (PB) in developing grains, before their maturity. PB are micrometer-sized organelles emerging from endoplasmic reticulum (ER) membranes, and may contain up to 80% of proteins[4]. An accumulation of proteins within the ER is suggested to explain the mechanism of PB formation. However, due to the absence of a retention signal in the sequence of these proteins, the sequestration and the accumulation processes of proteins in the lumen of the ER are unexplained. The aim of this study was to elucidate physical chemical parameters involved in the organization of WSP in the cellular context. In our study, the model WSP used were γ-gliadins and ω-gliadins. γ-gliadins are considered to be an ancestral form of storage proteins, and were shown to be able to alone form PB in heterologous systems.[5] ω-gliadins were also used because their involvement in PB formation could differ due to their fully repetitive sequence. This assumption is supported by the different surface properties of γ- and ω-gliadins at the air-water interface.[6] Furthermore, ω-gliadins should be considered as a model of the repetitive domain of γ-gliadins. First, lyotropic lamellar phases consisting of stacks of surfactant bilayers separated by aqueous layers were used as an ER model environment for gliadins. This 3D approach enabled us to study both the confinement effect on the protein assembly, and to suggest an interaction between gliadins and membranes. However, as the results obtained with this approach were not sufficient to firmly conclude on the gliadin-membrane interaction, we then used a 2D approach to look into protein

behavior at the water-lipid interface. In this case, DMPC and DMPG monolayers were used as ER model membranes. DMPC was chosen as it is the major phospholipid contained in plant ER membranes, while DMPG was selected to study the effect of the negative charges generally observed in biological membranes.

MATERIALS AND METHODS

Samples. γ- and ω-gliadins were purified according to the procedure of Banc et al.[6]. γ-gliadin was labeled with tetramethylrhodamine isothiocyanate (TRITC). The lamellar phase system was spontaneously formed with the nonionic n-pentaethyleneglycol monododecylether surfactant $C_{12}E_5$ (Nikko), hexanol and water. The hexanol/$C_{12}E_5$ weight ratio used was 0.29, and the water content varied from 40 to 90 wt% (i.e. repeat distances d from 50 to 180 Å). Three different probes were mixed with the lyotropic lamellar formulations at constant concentration [0.08% (w/w)]: rhodamine-labeled γ-gliadin (γ-gliadin-TRITC), rhodamine dye TRITC (mixed isomers, Aldrich) and hydrophobic probe, rhodamine 1,2-dihexadecanoyl-sn-glycero-3-phosphoethanolamine (DHPE-TRITC, Invitrogen-Molecular Probes). After mixing, samples were left for several days to reach equilibrium.

Determination of Diffusion Coefficients Using Fluorescence Recovery After Photobleaching (FRAP) Measurements. FRAP measurements were performed using a confocal laser scanning microscope (CLSM) that gives high spatial resolution and allows surgical bleaching. To measure the translational diffusion coefficient parallel to the layers ($D_\perp$ i.e. perpendicular to the optical axis), homeotropically oriented lamellar phases were used. In flat capillaries, the lamellar phase naturally adopted homeotropic anchoring (stacking axis perpendicular to the walls) and well-oriented monodomains were thus obtained. Bleaching and imaging were performed on a Leica SP2 confocal microscope with an oil immersion 363 objective lens. Data were analyzed using the Moreau et al. method.[7]

Measurement of Langmuir films. Experiments were conducted in a circular Teflon® trough with a 51-mm radius, filled with 8 mL of subphase. The subphase comprised a 50 mM pH 7.2 phosphate buffer previously filtered through a glass microfibre filter (GF/F, Whatman). Phospholipid monolayers were prepared with 1,2-Dimyristoyl-*sn*-Glycero-3-Phosphocholine (DMPC) or 1,2-Dimyristoyl-*sn*-Glycero-3-[Phospho-rac-(1-glycerol)] (DMPG) (Avanti Polar Lipids). Phospholipids were previously dissolved in chloroform (for DMPC), or chloroform:ethanol 4:1 (v/v) (for DMPG) at 0.5 mg/ml, and spread at the air-water interface using a Hamilton microsyringe. The volume injected was adjusted to obtain the required monolayer pressure. Freeze-dried proteins were dissolved in a 48:52 (v/v) water:ethanol mixture at a concentration C = 1 mg/mL, and filtered through a 0.45-µm porosity filter (Millipore). After equilibration of the phospholipid monolayer, protein solutions were injected into the subphase using a Hamilton microsyringe. The surface pressure (π) was monitored with a Wilhelmy surface balance using a filter paper plate (Whatman).

Brewster Angle Microscopy. The surfaces of the films were observed with a Brewster angle microscope BAM2 plus (NFT, Göttingen, Germany) equipped with a frequency doubled Nd:YAG laser with a wavelength of 532 nm and a charge-coupled device (CCD) camera with a x10 magnification lens. Depending on the image luminosity, exposure time (ET) was adjusted from 20 to 0.5 ms to avoid saturation of the camera. The spatial lateral resolution of the Brewster angle microscope was 2 µm, and the image size was 400 x 650 µm². The BAM images were coded in grey level and reflectance intensity versus thickness relationship was established.[8]

RESULTS AND DISCUSSION

3D approach: dynamic within the lamellar phases. Using FRAP experiments, the diffusion coefficient $D_\perp$ of each probe was measured as a function of the water layer thickness d_w of the $C_{12}E_5$/hexanol/water lamellar phase. The results obtained for the two probes (TRITC and DHPE-TRITC) and the protein (γ-gliadin-TRITC) are shown in Figure 1.

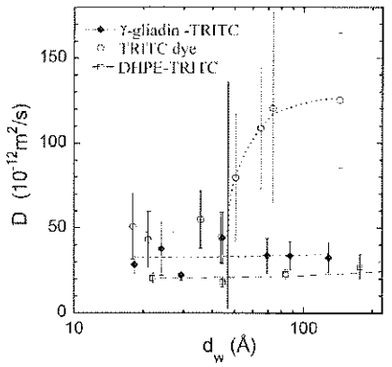


Figure 1: Diffusion coefficients $D_\perp$ derived from FRAP experiments as a function of the water layer thickness d_w for three different doped $C_{12}E_5$/hexanol/water lamellar phases: free TRITC (empty circles), γ-gliadin-TRITC (filled diamonds) and DHPE-TRITC (empty squares). The vertical solid line corresponds to d_w = 47 Å.

With the TRITC dye, the diffusion coefficient values were constant but the diluted – concentrated (or confined) transition occurred at a higher d_w value (47 Å, vertical full line in Fig. 1) than the expected hydrodynamic diameter of the TRITC dye (ca. 5 Å). TRITC could thus be present in an aggregated form within the aqueous layer of the lamellar phase. TRITC aggregation in water could also explain the large error bars obtained for diffusion coefficient from FRAP measurements. With the DHPE-TRITC probe, the diffusion coefficient was mostly independent of swelling (Fig. 1, empty squares). The values were low (20.10^{-12} m^2/s) and did not vary with confinement, behavior which is consistent diffusion of the amphiphilic probes along the bilayers. Interestingly, the same behavior was observed for the γ-gliadin-TRITC protein (Fig. 1, filled diamonds) encapsulated within the $C_{12}E_5$/hexanol/water lamellar phase. The diffusion coefficient value did not vary with d_w, and was equal to 30.10^{-12} m^2/s, again a low value. This invariance of the diffusion coefficient clearly showed that γ-gliadin did not freely diffuse in the water layer of the lamellar structure. Either the proteins were in interaction with the bilayers, or they formed large objects soluble in the aqueous medium which remained geometrically confined by adjacent bilayers in the whole range of d_w studied. In order to confirm and decipher a potential membrane-gliadin interaction, a complementary 2D approach was applied using different kinds of gliadins (γ- and ω-gliadins) and phospholipids (DMPC and DMPG).

2D approach: towards confirmation of membrane-gliadin interaction and consecutive gliadin self-assembly via the membrane interface. 2D experiments were carried out using DMPC or DMPG monolayers at the air-water interface, as model membranes. Figure 2 shows changes in interfacial pressure as a function of time with the injection of γ-gliadin solution under the DMPC monolayer. When the phospholipid monolayer had an initial pressure of 20 mN/m, injection of γ-gliadin caused an abrupt increase in interfacial pressure followed by stabilization at about 25 mN/m. This increase in pressure was ascribed to insertion of gliadins in the monolayer. When the DMPC monolayer had an initial pressure of 35 mN/m, even if a perturbation was initially observed, the surface pressure finally remained unchanged after a short equilibration period. At this higher pressure, γ-gliadins could not penetrate the DMPC monolayer. The same behavior was observed with the DMPG monolayer. Because the biological membrane pressure considered ranged from 25 to 35 mN/m,[9] we suppose that γ-gliadins did not penetrate the biological membranes. In this range of surface pressures, ω-gliadins exhibited the same behavior as γ-gliadins. Only the system with a 35 mN/m initial pressure was further characterized because our study focused on the behavior of gliadins in the ER lumen.

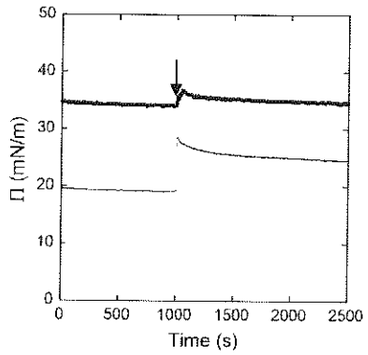


Figure 2: Changes in interfacial pressure P (mN/m) as a function of time (s) with the injection of γ-gliadin (arrow) under the DMPC monolayer at 20 mN/m (thin curve) and 35 mN/m (thick curve)

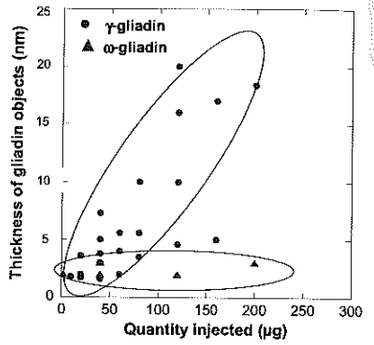


Figure 3: Thickness of gliadin objects (nm) as a function of gliadin quantity injected (µg). γ-gliadin (circles), ω-gliadins (triangles).

The effect of the injection of gliadins under the DMPC monolayer was observed by Brewster Angle Microscopy. At 35 mN/m, after the injection of gliadins under the DMPC monolayer, luminous dots appeared and disappeared, like blinking. This phenomenon was attributed to adsorption-desorption cycles of gliadins under the monolayer. After an equilibration period, the stabilized system displayed bright dots. Moreover, observation of the stabilized system by BAM with crossed polarizer and analyzer showed a dark picture, in accordance with the flat and smooth aspect of gliadin dots. When the quantities of protein injected were low, both γ- and ω-gliadins systems presented similar pictures, characterized by luminous dots with a micrometric radius. With higher quantities of gliadin, the γ-gliadin-DMPC monolayer system displayed more numerous larger and brighter dots than the ω-gliadin-DMPC monolayer system. Through the reflectance intensity of the dots – thickness relationship, we observed that the thickness of the γ-gliadin objects increased with the quantity of protein injected, whereas the thickness of the ω-gliadin objects remained constant (Figure 3). The average thicknesses corresponding to the dots reflectance ranged between 8 and 20 nm in the presence of γ-gliadins and were about 6 nm in the presence of ω-gliadins. Considering the thickness (2.2 nm) of the DMPC monolayer, the thickness of the γ-gliadin aggregates increased from approximately 6 to 18 nm with the addition of proteins, whereas the thickness of the ω-gliadins aggregates remained constant at approximately 4 nm. With low quantities of γ- and ω-gliadins injected, the thickness of the aggregates suggested gliadin monolayers adsorbed under the DMPC monolayer. With high quantities of γ-gliadins injected, but not ω-gliadins, the calculated thickness suggested a multilayer structure of the protein aggregates. BAM observations of systems made with DMPG monolayers were qualitatively the same as those performed using DMPC monolayers. We also observed the formation of flat, circular, micrometric gliadin aggregates under the lipidic monolayer.

BAM observations indicated that both γ and ω-gliadins locally adsorbed under lipid monolayers to form small domains with limited lateral expansion. This behavior suggested a nucleation-growth mechanism for the formation of gliadin aggregates under the membrane. The limited lateral growth was attributed to an important line tension between the adsorbed gliadin aggregates and the subphase molecules in interaction with polar heads of phospholipids. The thickness of these domains differed depending on the type of gliadin. Initially, γ- and ω-gliadins adsorbed as monolayers under the lipidic monolayer. With increasing quantities of gliadins injected into the subphase, the γ-gliadins aggregate thickness increased whereas with ω-gliadins, aggregate thickness remained constant. Considering that γ-gliadins displayed an amphiphilic structure comprising a hydrophobic, non-repetitive domain, and a more hydrophilic, repetitive domain, and that ω-gliadins were mainly composed of a repetitive domain, we propose a model to explain our experimental observations (Figure 4). The interaction between protein and membrane, observed using both γ- and ω-gliadins, could be ascribed to an interaction between polar heads of phospholipids and the

repetitive domain of gliadins through the establishment of intermolecular H bonds between lipid bilayers and gliadins.

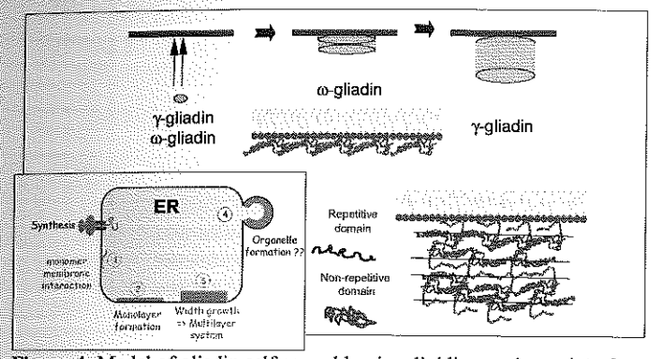


Figure 4: Model of gliadin self-assembly via a lipidic membrane interface. The formation of a multilayer structure of γ-gliadins under the lipid membrane would occur through mainly hydrophobic interactions (through non-repetitive domains) while the formation of a ω-gliadin multilayer under the lipid membrane would be inhibited due to the absence of hydrophobic domains within the sequence.

CONCLUSIONS

The demonstration of γ- and ω-gliadin-membrane interaction, using a combined 3D approach using lamellar phases and a 2D approach using Langmuir phospholipid films, could explain the initiation of prolamin assembly within the endoplasmic reticulum of wheat endosperm cells. Moreover, the increase in aggregate thickness observed in our prolamin-membrane model systems could be a mechanism in the accumulation process that occurs in the biological context. A model of the accumulation process based on the amphiphilic nature of γ-gliadins is proposed (Figure 4). Further studies will be needed to confirm this hypothetical model of prolamin accumulation from a membrane, and in particular by better characterization of gliadin aggregates using techniques that provide local information such as AFM and microspectroscopy.

REFERENCES

1. Sliwinski, E. L., Kolster, P., Prins, A., van Vliet, T. 2004. On the relationship between gluten protein composition of wheat flours and large-deformation properties of their doughs. J. Cereal Sci. 39: 247–264.
2. Pallos, F., Robertson, G., Pavlath, A., Orts, W. 2006. Thermoformed wheat gluten biopolymers. J. Agric. Food Chem. 54: 349–352.
3. Orecchioni, A. M., Duclairoir, C., Renard, D., Nakache, E. 2006. Gliadin Characterization by Sans and Gliadin Nanoparticle Growth Modelization. J. Nanosci. Nanotcchnol. 6: 3171-3178.
4. Galili, G., Shimoni, Y., Giorini-Silfen, S., Levanony, H., Alschuler, Y., Shani, N. 1996. Wheat storage proteins: Assembly, transport and deposition in protein bodies. Plant Physiol. Biochem. 34: 245–252.
5. Galili, G., Altschuler, Y., Levanony, H., Giorinislfen, S., Shimoni, Y., Shani, N., Karchi, H. 1995. Assembly and transport of wheat storage proteins. J. Plant Physiol. 145: 626-631.
6. Banc, A., Desbat, B., Renard, D., Popineau, Y., Mangavel, C., Navailles, L. 2007 Structure and Orientation Changes of ω- and γ-Gliadins at the Air-Water Interface: A PM-IRRAS Spectroscopy and Brewster Angle Microscopy Study. Langmuir 23: 13066–13075.
7. Moreau, P., van Effenterre, D., Navailles, L., Nallet, F., Roux, D. 2008 Confined diffusion of hydrophilic probes inserted in lyotropic lamellar phases. Eur. Phys. J. E 26: 225–234.
8. Banc, A., Desbat, B., Renard, D., Popineau, Y., Mangavel, C., Navailles, L. 2009 Exploring the interactions of gliadins with model membranes: effect of confined geometry and interfaces. Biopolymers 91: 610-622.
9. Rakotomanga, M., Loiseau, P.M., Saint-Pierre-Chazalet, M. 2004 Hexadecylphosphocholine interaction with lipid monolayers. BBA-Biomembranes 1661: 212-218.