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Gliadin effect on giant vesicle elastic modulus

Abstract Wheat gluten consists of a complex supramolecular protein assembly of very high molecular mass, with over a million daltons, that displays important viscoelastic and interfacial properties. Its involvement in an immunologically based nutritional disease, the celiac disease, is thought to be somehow related to nontrivial structural features of the protein complex, apparently involving gliadin, one of its fractions. Despite its medical relevance, the involvement of gliadin in the etiology of the celiac disease is nevertheless not understood in terms of the mechanisms involved. In this context, gliadin was investigated relative to its effect on fluid bilayer elasticity. Dioleoylphosphatidylcholine giant unilamellar vesicles (about 50-μm diameter) were produced by electric field pulsing over metal-covered plates. The technique of micropipette suction allowed vesicle invagination into a micropipette by applying a suction pressure. A video camera coupled to a microscope permitted the suction process to be monitored and the different relevant geometric quantities to be measured: vesicle projection length into the pipette, for different applied pressures; vesicle and pipette diameters. The vesicle projection length into the pipette as a function of pressure, for a given vesicle and pipette diameter, is related to the bilayer bending modulus k_c. Assayed samples comprised vesicles with and without ethanol-dissolved gliadin incorporated in the membrane, at two distinct gliadin/phospholipid mass fractions. We observed a significant increase in k_c of the phospholipid bilayer with the largest gliadin content (0.02 gliadin/dioleoylphosphaditylcholine w/w), from about $k_c = 21\ k_BT$, to $k_c = 52\ k_BT$, where k_BT is the thermal energy of the solution. The results reveal the affinity between the protein and the bilayer, suggesting possible correlations between membrane mechanical properties and the celiac disease etiology.

Keywords Gliadin · Bending modulus · Celiac disease · Gluten · Giant vesicles

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Introduction

Gliadin is a component of wheat gluten, a complex supramolecular protein assembly of very high molecular mass [1]. Besides the importance of gluten in biotechnology, owing to its viscoelastic properties and interfacial activity, it is also known to be involved in a disease of autoimmune character. The condition, known as the celiac disease, comprises an intolerance to gluten ingestion and is characterised by remarkable histological changes of the intestinal mucosa involving the complete loss of its microvillosities and their conversion into a

smooth, flattened surface. Such changes lead to the failure of the mucosa absorption capacity, with devastating consequences for the nutritional status of the patient [2]. Neurological implications of the disease have also been more recently reported in the literature [3].

Considering the peculiar changes displayed by the celiac intestinal mucosa, we investigated possible alterations induced by gliadin on mechanical properties of a model cell membrane. In particular, we determined the effect of gliadin on the bending elasticity of dioleoylphosphaditylcholine (DOPC) vesicles. The study revealed a sizeable dependence of the membrane rigidity on protein concentration. Our results point to the importance of accounting for membrane elasticity modifications when the etiology of the celiac disease is studied.

Methodology

Vesicles in a solution exhibit thermal fluctuations due to Brownian motion [4, 5]. A micropipette apparatus, first developed by Evans [6, 7], measures the relation between the excess area stored in the fluctuations and the corresponding value of the membrane tension [6]. This relation, known as the Helfrich law, provides for the determination of the membrane bending modulus k_c. In practice one determines α, the relative amount of the membrane surface stored in the fluctuating modes and the membrane tension σ by measuring both the geometrical parameters related to the invaginated vesicles and the aspiration pressure. From the Helfrich relation [8],

$$\alpha = \frac{A^a - A_0^a}{A_0^a} = \frac{k_B T}{8\pi k_C} \ln\left(\frac{\sigma}{\sigma_1}\right), \tag{1}$$

the relation of the excess area, α, and the bending modulus, k_c is established.

In Eq. (1), A_0^a and A^a are the initial and the final apparent projected areas, σ_1 is the value of the first measured tension, T is the absolute temperature and k_B is the Boltzmann constant. The apparent areas in Eq. (1) can be experimentally estimated by measuring parameters R_0, R_L and r, as indicated in the schematic drawing in Fig. 1.

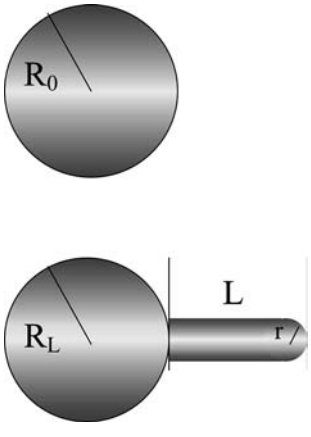

Fig. 1 Schematic drawing indicating parameters analyzed for the vesicles in the micropipette suction experiments

The experimentally measured parameters, indicated in the schematic drawing in Fig. 1, are related to the apparent areas by simple geometrical relations (Eqs. 2, 3):

$$A^a = 4\pi R_L^2 - \pi r^2 + 2\pi r(L - r) + 2\pi r^2, \tag{2}$$

$$A_0^a = 4\pi R_0^2. \tag{3}$$

The excess area is therefore given by

$$\alpha = \left[\frac{R_L^2 + (\frac{rL}{2}) - (\frac{r^2}{4})}{R_0^2}\right] - 1 \tag{4}$$

which allows the determination of k_c [8, 9].

In this work, we were particularly concerned in obtaining mechanical information relative to k_c of a model membrane system, as affected by the presence of gliadin, by means of the Evans micropipette technique. With that purpose, DOPC giant vesicles with an average diameter of 50 μm were produced by electroformation [4]. Phospholipid films were prepared from DOPC dispersed in chloroform, followed by vacuum drying overnight. Samples containing gliadin (whole gliadin fraction from wheat gluten, a Sigma-Aldrich product) were prepared by addition of ethanol-dispersed gliadin to the DOPC/chloroform mixture. A sucrose solution was used to hydrate the phospholipid films during vesicle formation. Transference of vesicles to a glucose solution of same osmolarity provided for the vesicle integrity and for the necessary visual contrast for microscopic observation.

Results and discussion

The micropipette technique devised by Evans and collaborators has proved very relevant in investigations of surface and micromechanical aspects of model surfaces in several works undertaken during the last decade. Examples include studies of cell adhesion and microtubular formation [10], elaboration of membrane rupture models through the investigation of spontaneous frequencies of defect formation and its correlation with membrane thickness and bending modulus [11], investigation of membrane water permeability [12] and studies on the dependence of bilayer elasticity on lipid chain length and degree of unsaturation [13], among others.

The results in the present work indicating the variation of α as a function of the logarithm of the membrane tension σ, for DOPC vesicles containing gliadin at two different concentrations and for a blank sample of pure DOPC, are displayed in Fig. 2. The values of k_c determined and their respective standard deviations are shown in Table 1.

Table 1 Effect of wheat gliadin on the bending modulus, k_c, of dioleoylphosphatidylcholine (*DOPC*) giant vesicles

Sample	k_c/k_BT	Standard deviation
(1) gliadin/DOPC 0.01 (w/w)	32.80	14.23
(2) gliadin/DOPC 0.02 (w/w)	52.16	18.86
Blank sample (1)	21.59	4.50
Blank sample (2)	20.22	3.68

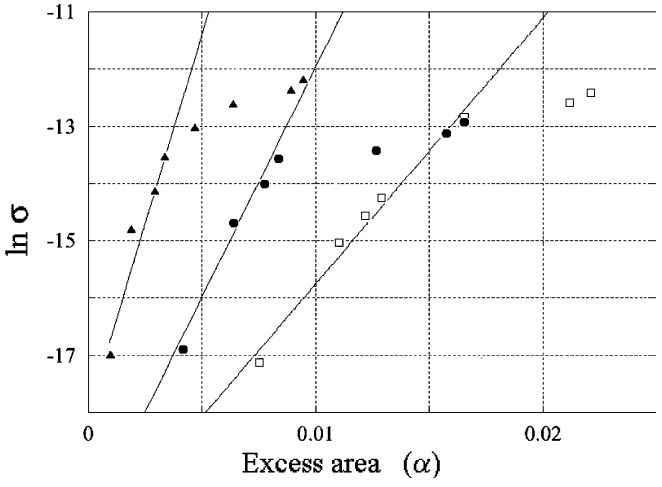

Fig. 2 Variation of the logarithm of the tension σ as a function of the excess area α: blank sample (vesicle of pure dioleoylphosphatidylcholine, *DOPC*) (*squares*); vesicle made of 99% DOPC + 1% w/w gliadin (*circles*); vesicle made of 98% DOPC + 2% w/w gliadine (*triangles*)

According to Eq. (1), the ascending linear region on the graph, attained for the smallest tension values, is the region of interest for assessing the value of k_c. The average values for ten experiments show an increase in k_c from around 21 k_BT for the vesicles of pure DOPC to 32.80 and 52.16 k_BT for protein-containing vesicles at 1% and 2% protein concentration, respectively (Table 1). These results point to the affinity of gliadin for the vesicle bilayer, as expected for a protein with highly hydrophobic character. It is clear that changes in the bending modulus of phospholipid membranes must be a reflection of structural changes of the phospholipid bilayer induced by its interaction with the protein. As an hydrophobic entity, gliadin is likely to penetrate the lipophilic membrane core, where it may change patterns of lipid ordering and flexibility. Changes of around 10 k_BT have been reported by Evans and Rawicz [14] for the bending rigidity of membranes in the presence of grafted water-soluble polymers in the semidilute regime, with the membrane stretching modulus remaining unaltered. A considerably more pronounced effect on the bending modulus is verified for membrane k_c in the presence of minor amounts of gliadin (1–2% relative to the DOPC content), as reported in this work. Considering the pronounced hydrophobic character of that protein and peculiarities of its three-dimensional structure, as revealed in modelling work [15, 16], we argue that its interaction with DOPC should involve protein penetration into the apolar region of the bilayer, rather than a simple anchoring on its external surface. Membrane mechanical properties may be affected by the peculiar viscoelastic behaviour of the interacting protein. Permeation across the bilayer might present alterations as well, leading ultimately to significant morphological changes of the membrane. In an in vivo context, where a specific immunological response takes place, these modifications may contribute to the observed morphological and functional changes in surface properties, such as those displayed by the celiac mucosa.

Conclusions

Gliadin showed an effect of increasing k_c of the phospholipid bilayer in giant vesicles of DOPC. Such an effect was noticeable for minor amounts of the protein present in the preparations (0.01–0.02 w/w protein-to-DOPC ratio). Results discussed on the basis of gliadin structural and mechanical properties suggest possible correlations between changes in elasticity and aspects of the etiology of the celiac disease.

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