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Binding of Gliadin to Lymphoblastoid, Myeloid and Epithelial Cell Lines

M.A. FARRÉ CASTANY[a], P. KOCNA[b], and H. TLASKALOVÁ-HOGENOVÁ[a]

[a]*Department of Immunology and Gnotobiology, Institute of Microbiology, Academy of Sciences of the Czech Republic, 142 20 Prague 4, Czech Republic*
[b]*Laboratory of Gastroenterology, Faculty of General Medicine, Charles University, 121 11 Prague 1, Czech Republic*

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ABSTRACT. The aim of our work was to investigate the *in vitro* reactivity of gliadin peptides of natural and synthetic origin with various cell lines. We have found that all tested cell lines of human, mouse and rat origin were agglutinated by enzymically digested gliadin (peptic–tryptic- and peptic–tryptic pancreatic digest of α-gliadin) in a concentration dependent manner. In order to test the specificity of binding, inhibition studies were performed using a panel of sugars as well as natural and synthetic peptides derived from gliadin. We have found that among twelve tested sugars only fetuin and phosphomannan were able to inhibit the agglutination of K562 cells with peptic–tryptic- but not with peptic–tryptic pancreatic digest of α-gliadin. The lack of inhibition by gliadin peptides and most of the saccharides suggests that agglutinating activity of gliadin is the result of a nonspecific binding of gliadin to the cell membrane.

Abbreviations

FFIII	Frazer's fraction III
PTP	peptic-tryptic- pancreatic digest of α-gliadin
PBS	phosphate buffered saline
BSA	bovine serum albumin
ConA	concanavalin A

The mechanism by which gluten (the triggering agent in celiac disease) affects the small intestinal mucosa is still unclear. Weiser and Douglas (1976) proposed that gluten contains a toxic lectin which binds to altered membrane glycoproteins of celiac enterocytes. This hypothesis was supported by Köttgen *et al.* (1982) who confirmed that gluten has lectin-like properties and that gluten peptides can bind to glycoproteins present on immature crypt cells of rat intestine and in human serum. It has also been reported that gliadin binds to B lymphocytes of celiac patients (Verkasalo 1982), T lymphocytes (Payan *et al.* 1987), and to reticulin in tissue sections (Unsworth 1987). Auricchio *et al.* (1984) reported that peptic–tryptic digest of bread wheat gliadin and A-gliadin agglutinated the undifferentiated K562 human cell line of myeloid origin, which is used as NK cell target in assays measuring spontaneous cytotoxicity.

The purpose of the present study is to investigate the agglutination of cell lines of different origin by gliadin peptides, and to define the nature of gliadin binding to cell membrane. Cells of lymphoblastoid, myeloid and epithelial lineages were used in agglutination assay employing peptides derived from gliadin. Studies of inhibition of the agglutination activity were performed on K562 cells using synthetic peptides and various saccharides. Moreover, we evaluated the binding of gliadin to the colon epithelial cell line (HT-29) using the biotin–streptavidin system.

MATERIALS AND METHODS

Gliadin preparations. Crude gliadin (*Sigma Chemical Co.*, St. Louis, MO), α-gliadin, β-gliadin and γ-gliadin (gliadin fractions characterized by its electrophoretic mobility) were first dissolved in 70 % ethanol and then in PBS. A-Gliadin, Frazer's fraction III (*i.e.*, peptic–tryptic digest of crude gliadin), and peptic–tryptic pancreatic digest of α-gliadin (PTP) were dissolved in PBS. All gliadin peptides were prepared as described earlier (Stoyanov *et al.* 1988).

Synthetic peptides. Four dodecapeptides derived from α-gliadin were prepared by multiple continuous-flow solid-phase peptide synthesis (Vágner *et al.* 1991) and kindly provided by Dr. V. Krchňák from the *Research Institute for Feed Supplements and Veterinary Drugs*, Jílové (Czech Republic). The amino acid sequences of synthetic peptides were as follows:

P-8-7	L Q P Q N T G Q Q Q P Q
P-14-9	L Q P Q N P S A A A P Q
P-20-3	L Q P Q N P S P P P P Q
P-20-4	L Q P Q N P S N N N P Q

Saccharides. Different saccharides and their derivatives were used for the inhibition studies. N-Acetyl-D-galactosamine, N-acetyl-D-glucosamine, D-glucosamine, phosphomannan, D-fucose, α-D-glucose, D-galactose, mannan, D-mannose, D-mannitol and lactose were from *Lachema*, Prague; fetuin was from *Sigma Chemical Co.*, St. Louis (MO). All these saccharides are competitive for different lectinic bonds.

Cell lines. We used the following cell lines:

1 – of human origin: K562 chronic myelogenous leukemia, RAJI B-lymphoblastoid cells (Burkitt's lymphoma of maxilla) and HT-29 colonic adenocarcinoma cells (epithelial);

2 – of mouse origin: YAC-1 AS/N with Moneti virus, DCH-5 dendritic cells, EL-4 lymphoma cells, S2 and RAW macrophages, L929 fibroblasts. The mouse teratocarcinoma cells P19X1 and P19S1801A1, and their derivative mutants (P19XT.1.1, P19ST.1.3 and P19ST.1.5) which are defective in some (P19ST.1.3 and P19ST.1.5 cells) or all (P19XT.1.1 cells) of the three following sugar epitopes: D-fucose, D-galactose and N-acetyl-D-galactosamine (Dráber *et al.* 1987) were kindly provided by Dr. Dráber from the *Institute of Molecular Genetics*, Prague;

3 – of rat origin: RBL-2H3 macrophages was a gift from Dr. Dráberová.

Agglutination assay. The agglutination test was performed similarly to Auricchio *et al.* (1984). Briefly, a volume of 25 μL of cells suspended in PBS ($3-5 \times 10^5$) was applied to each well of a 96-well microtiter plate (*Hardtmuth Koh-i-noor*, České Budějovice, Czech Republic) containing 100 μL of gliadin peptides in 2-fold dilutions starting from 500 or 100 mg/L for natural and synthetic peptides, respectively. Cell suspension containing 100 μL (20 mg/L) of ConA (*Sigma Chemical Co.*, St. Louis, MO) or PBS was used as positive and negative control, respectively. Microplates were incubated for 30 min at room temperature and the agglutinating activity was evaluated microscopically.

In order to study the specificity of the binding, two natural peptides: FFIII (3.5 mg/L) and PTP (7.5 mg/L) at subagglutinating doses, five synthetic peptides (30 mg/L), and twelve sugars (2 mmol/L) were used in a competitive agglutination assay. The inhibition was performed as follows: cell lines were incubated with the inhibitor for 1 h at 37 °C, washed with PBS and then assayed with decreasing concentrations of gliadin peptides as described above.

ELISA assay for detection of gliadin binding to cells. For this purpose biotinylation of FFIII was made using a solution of biotinyl-N-hydroxysuccinimide prepared in dimethyl sulfoxide (*Boehringer* Mannheim, Germany) (Pescovitz *et al.* 1985). Briefly, 120 μg of biotinyl ester (1 g/L) was incubated with 1 mg of FFIII (1 g/L) for 4 h at room temperature and dialyzed against PBS. For ELISA test, HT-29 epithelial or K562 lymphoblastoid cell lines (5×10^4 cells per well) were applied into 96-well microtiter plates and incubated overnight at 4 °C. After blocking with 1 % ovalbumin in PBS for 30 min at room temperature, the cells were fixed for 10 min with 0.1 % glutaraldehyde. After several washings, 50 μL of biotinylated gliadin (FFIII) diluted in PBS was added in duplicate at decreasing concentrations (100, 75, 50, 25, 5, 1 and 0.25 mg/L), and incubated for 1 h at 37 °C. Wells filled with PBS only were used as negative control. After washing, 50 μL streptavidin conjugated to peroxidase (*Immunotech*, Marseille) diluted 1:1000 in BSA 1 % was added and the plates were incubated for 1 h at 37 °C. After several washings, the enzymatic reaction was developed by 1,2-benzenediamine solution, stopped by 2 mol/L H_2SO_4, and read on the Microelisa MR 500 Minireader (*Dynatech*) at 470 nm.

In inhibition experiments, 50 mg/L of non-labeled FFIII was applied to glutaraldehyde-fixed cells. After 1 h of incubation at 37 °C, biotin conjugated to FFIII was added.

RESULTS

Gliadin binding assessed by agglutination

All cell lines of lymphoblastoid, myeloid and epithelial origin were agglutinated by two enzymically digested peptides of α-gliadin (FFIII and PTP) in a concentration dependent manner. The minimal concentration of gliadin needed to agglutinate each cell line varied substantially: most of the cell lines tested were found to be agglutinated by a low amount of gliadin; only teratocarcinoma cells required higher amounts of FFIII and PTP (Table I).

Table I. Minimum concentration of FFIII and PTP (mg/L) causing agglutination of different cell lines

Cell line	Origin	Gliadin peptides[a]	
		FFIII	PTP
K562	HUMAN	7.5	7.5
RAJI		7.5	31
HT-29		15.5	15.5
YAC-1	MURINE	15.5	15.5
DCH-5		7.5	7.5
EL-4		7.5	–
S2		7.5	–
RAW		0.4	–
L929		3.25	–
P19X1		62.1	31
P19XT.1.1		15.5	125
P19ST.1.3		62.5	15.5
P19ST.1.5		125	62.5
RBL-2H3	RAT	3.25	0.8

[a]Added in 2-fold dilution; starting concentration was 500 mg/L. In all experiments ConA (20 mg/L) was used as positive control of agglutination.

The myelogenous and lymphoblastoid K562, RAJI, YAC-1 and RBL-2H3 cells which were also tested in agglutination assays using α-, β-, γ- and A-gliadin did not react with any of the two electrophoretic α- and β-gliadin fractions (Table II). Synthetic peptides derived from α-gliadin in a concentration of 100 mg/L were also unable to agglutinate K562 cells (*data not shown*).

Gliadin binding assessed by ELISA

In the ELISA test for estimation of gliadin binding to HT-29 and K562 cells we found that FFIII was able to bind to both lines in a concentration dependent manner. Starting from 5 mg/L we observed substantially higher gliadin binding to myelogenous K562 cells as compared with HT-29 epithelial cells (Fig. 1).

Inhibition studies

In inhibition studies performed on the K562 cell line, none of the gliadin-derived synthetic peptides used at 30 mg/L inhibited the agglutination of these cells induced by PTP in two subagglutinating concentrations (7.5 and 30 mg/L). Similarly, FFIII (3.5 mg/L) and PTP (7.5 mg/L) where not able to inhibit the agglutinating activity of these cells when tested with different concentrations of the same peptide (*data not shown*).

Table II. Minimum concentration of gliadin peptides (mg/L) causing agglutination of K562, RAJI, YAC-1 and RBL-2H3 cell lines

Gliadin peptide[a]	Cell line			
	K562	RAJI	YAC-1	RBL-2H3
α-Gliadin	>500	>500	>500	>500
β-Gliadin	>500	25	>500	>500
γ-Gliadin	6.25	25	>500	>500
A-Gliadin	6.25	6.25	>500	>500
Crude gliadin	3.12	>500	>500	0.4

[a]Added in 2-fold dilution; starting concentration was 500 mg/L. In all experiments ConA (20 mg/L) was used as positive control of agglutination; ≤500 mg/L: agglutination was not observed.

When we performed the inhibition with a range of sugars, we found that only fetuin and phosphomannan were active and inhibited the agglutinating activity of K562 cells with FFIII but not with PTP at the concentration 30 mg/L (Table III).

The inhibition of binding of biotinylated gliadin to HT-29 and K562 cells using unlabeled proteins was also unsuccessful for both cell lines (*data not shown*).

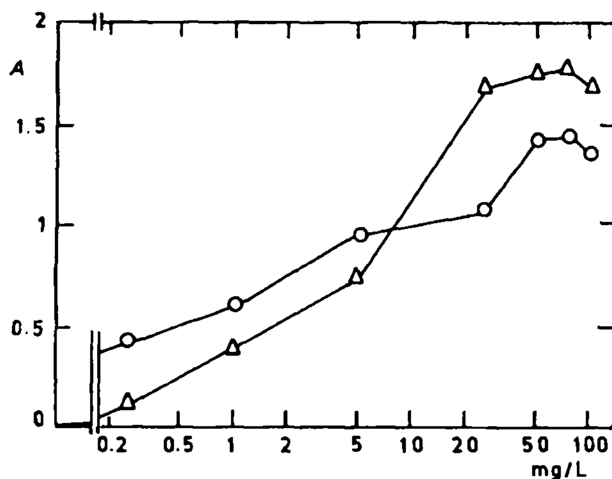

Fig. 1. Gliadin binding (absorbance *A*) to HT-29 epithelial (*circles*) and K562 myelogenous (*triangles*) cell lines at increasing concentrations of FFIII (mg/L).

DISCUSSION

The question of the lectin-like behavior of gluten in celiac disease remains a controversial issue. Several investigators support the hypothesis that gliadin contains a lectin which binds to intestinal cells damaging them (Kolberg *et al.* 1985, 1987; Auricchio *et al.* 1987, 1990). Several authors suggest a non lectin-mediated interaction of gliadin peptides with human and rat enterocytes (Colyer *et al.* 1987). Moreover, Pittschieler *et al.* (1994) showed that lectin binds to intestinal mucosa of celiac patients in a non-lectin-like binding of gliadin with enterocytes. The lectin hypothesis of celiac disease has also been questioned by Ruhlmann *et al.* (1993) who did not find correlation in the binding of gliadin peptides to glycoproteins. Moreover, competitive saccharides were unable to inhibit the binding.

In this study we report on several lines of evidence which support the non lectin-like binding of gliadin peptides to cell membrane. We found that natural peptides derived from gliadin by enzymic treatment agglutinate all tested cell lines, even teratocarcinoma cell mutants which are saccharide deficient. We observed that ethanol-soluble α- and β-gliadin were not able to agglutinate cell lines at the tested concentrations (Table I, II). We assume that the insolubility of peptides in water may cause their inability to interact with cells. Unsworth *et al.* (1993) published an interesting result showing that enzyme solubilized gliadin (FFIII) but not undigested wheat gluten activated complement in human sera of normal and celiac patients.

It seems that the type of enzymic treatment of gliadin and the resulting peptides length can also affect the inhibition of agglutination of K562 cells by fetuin and phosphomannan.

Table III. Effect of various saccharides on agglutination of K562 cells by gliadin[a]

Saccharides 2 mmol/L	FFIII 30 mg/L	PTP 30 mg/L
N-Acetyl-D-glucosamine	+	+
N-Acetyl-D-galactosamine	+	+
D-Glucosamine	+	+
D-Glucose	+	+
D-Galactose	+	+
D-Mannose	+	+
D-Mannitol	+	+
D-Fucose	+	+
Lactose	+	+
Mannan	+	+
Phosphomannan	−	+
Fetuin	−	+

[a] +, agglutination; −, inhibition.

Our results thus support the recent findings of Osman *et al.* (1994) who described a non-selective association of gliadin with a variety of different proteins. Casein and major proteins (fibrinogen, fibronectin and mucin) were shown to bind strongly to gliadin in a non-specific way, probably by hydrophobic interaction. Our data also suggest that the agglutination of cells with gliadin is mediated by a non-lectin interaction.

Recently, immunopathological mechanisms were suggested as the main cause of celiac disease. From our results it cannot be excluded that nonspecific gliadin binding could result in triggering immune responses directed to self structures. *In vivo* gliadin binding to different cells could precipitate an inflammatory response which could lead to an intestinal damage.

In our previous studies we have shown that gliadin can activate lymphocytes of celiac patients (Tlaskalová-Hogenová *et al.* 1990). More recently we have published the possible structural similarities between gliadin and self-components which were recognized by antigliadin antibodies, and could thus generate autoimmune reactions (Tučková *et al.* 1995). However, it is not excluded that nonspecific binding of gliadin to membrane proteins of various cell types could result in a profound change in their biological functions.

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