ISOLATION AND PROPERTIES OF A LECTINLIKE GLYCOPEPTIDE FROM WHEAT GLUTEN

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INTRODUCTION

Coeliac disease (gluten-sensitive enteropathy) is characterized by damaged jejunal mucosa which improves in the absence of wheat gluten and some other prolamines from the diet. The mechanism of the toxic action of gluten, gliadins, and their proteolytic fragments and the true pathogenesis of the coeliac disease remain unexplained. Various mechanisms of the etiology and pathogenesis have been suggested. One of these hypotheses, a mechanism proposed by Wieser in 1976 (1) is that gluten (or gliadin) is bound in lectinlike manner to pathologically altered glycoproteins on the enterocyte surface. This alteration may be genetically determined. A variety of experimental approaches have been reported to support this hypothesis (3–5, 9, 10) although conflicting results were also described (6–8). Immunological aspects should be taken into account as immunological mechanisms are probably involved in the pathogenesis of coeliac disease too.

The binding of gluten is enabled by the presence of defined oligosaccharide unit of the glycoprotein and Köttgen (3) reported that all gluten-reactive glycoproteins possess the high-mannose type of glycosylation. This reactivity with some proteins can be abolished by enzymatic cleavage with endoglycosidase H (3) and a 50% inhibition of the gluten binding has been found for mannan (a high mannose glycopeptide) at a 10μM concentration. In keeping with the pathogenesis of coeliac disease Köttgen (3) found the strongest gluten binding to glycoprotein from immature cryptic cells with gluten, while that from the mature villous zone shows only a minimal reaction. Mannan exhibited a protective effect and allowed morphological improvements of small bowel mucosa from coeliac patients cultivated in the presence of gliadin peptides (10). Mannan very effectively inhibits agglutinating activity of prolamine peptides (5) and is able to protect fetal intestine mucosa from in vivo effects of all the toxic prolamine peptides (10). Recently, Köttgen described an in vivo induction of gliadin-mediated enterocyte damage in rats by the mannosidase inhibitor swainsonine (11). This inhibits α-mannosidase II and causes the synthesis of hybrid-type glycoprotein.

Douglas in 1976 isolated a glycopeptide from wheat gluten called ''glyc-gli'' (2), that binds to the intestinal mucosa of coeliac patients but not of control subjects. This glycopeptide has been

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tested by Concon (4) who found a high lectin activity in it. Toxicity of this gluten preparation was confirmed by challenge tests with 500 mg/day and the binding studies to intestinal mucosa were carried out by using ^{125}I-labelled material (2).

MATERIALS AND METHODS

We prepared the glyc-gli glycopeptide according to Douglas (2) by trichloracetic acid precipitation of the ethanol–acetic extract of the crude gluten in the amount of 800 mg. By the SDS-PAGE in 10% polyacrylamide gel the main fraction with molecular mass 15 400 and some additional high-molecular-weight fractions were found. A considerable heterogenity of pI across the whole range of pH 3.5–9.5 was demonstrated by isoelectric focusing. Compared with other gliadin preparations, only in glyc-gli were these protein bands in the acidic area (pH 4–6). *Figure 1* demonstrates the analysis of glyc-gli by gel-permeation chromatography (GPC) using the automated HPLC apparatus Perkin–Elmer (12) on the TSK G2000-SW column eluted with 0.1% trifluoroacetic acid in 0.25 M sodium chloride at a flow of 0.4 ml/min. The molecular mass estimated by this method was found to be 10 000 for the main peak.

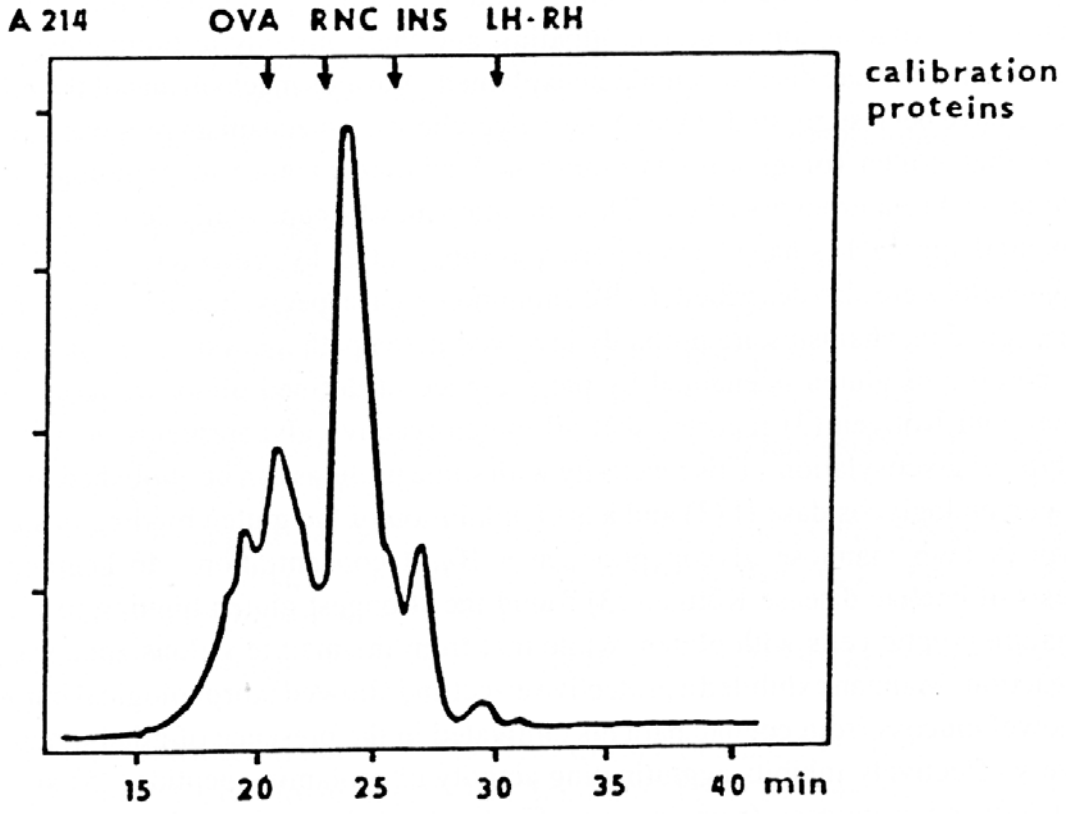

Figure 1. Analysis of glyc-gli using GPC method. The column 7.5 x 300 mm TSK G2000-SW was eluted with 0.1% TFA 0.25 M NaCl at 0.4 ml/min, detection UV 214 nm. Retention times of calibration proteins (ovalbumin OVA, ribonuclease RNC, insulin INS and LH-RH) are marked.

The ELISA method was used to determine the reactivity of antibodies present in sera of patients with coeliac disease to glyc-gli as well as to compare their reactivity with other gliadin preparations. α-, β-, γ-, and ω-gliadins were prepared by ion-exchange chromatography on Sulfopropyl-Sephadex C-50 according to Charbonnier (13, 14), aggregated A-gliadin was from

State University at Leiden and Frazer F-III fraction was prepared from gluten by standard method (15). ELISA tests were performed as described earlier (16, 17) and the different gliadin preparations were fixed to polystyrene microtiter plates with 10% formaldehyde solution. After incubation with the sera of patients, specific anti-IgA and anti-IgG antibodies conjugated with peroxidase were added and the activity was measured by Microelisa Minireader MR 500 (Dynatech).

RESULTS AND DISCUSSION

Figure 2 demonstrates the antibody activities found in the sera of coeliac patients and healthy control subjects directed against different gliadin preparations. A significant difference between antibody activities in sera of coeliac patients and control subjects were found mainly in IgG isotype. Correlation coefficients were then calculated for both IgA and IgG antibodies, comparing each gliadin preparation with each of the others, using individual values for each subject. Correlation between IgA and IgG antibodies was not found, but high significant correlations of coupled gliadin preparations, mainly in case of IgA antibodies in coeliac patients were found. In the control group the correlation coefficients were found to be very low (Table I).

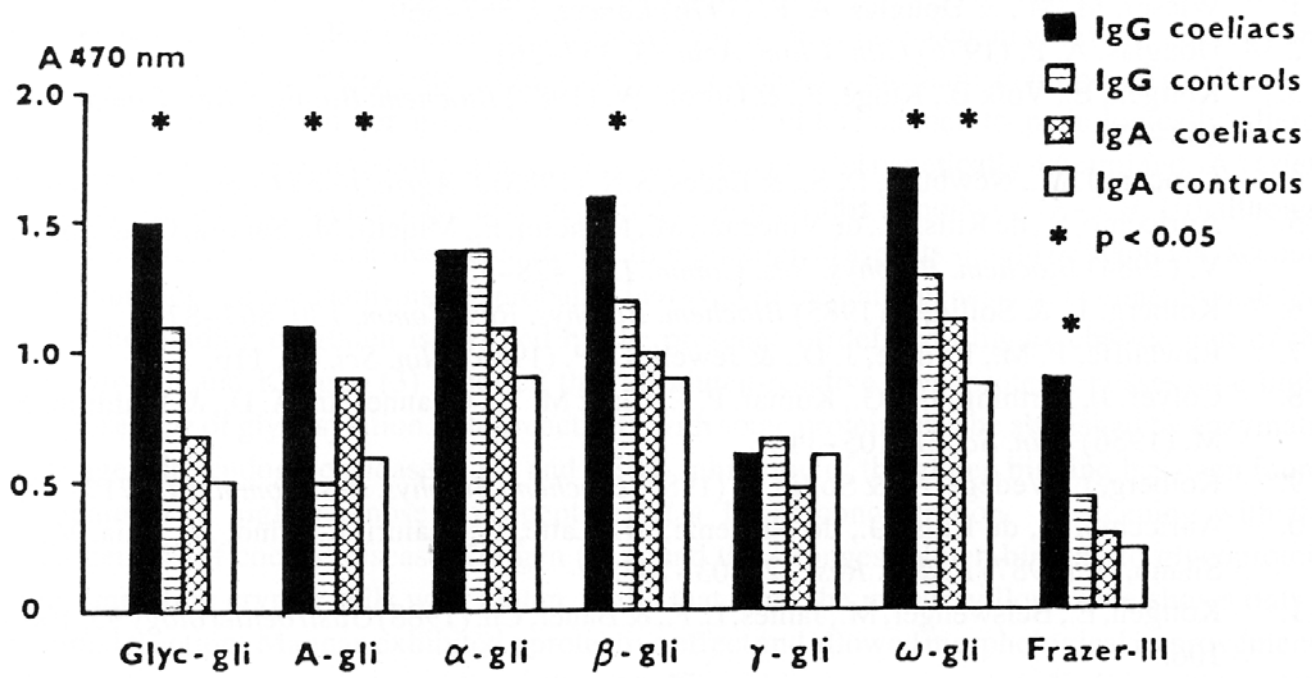

Figure 2. Reactivity of IgA and IgG antibodies in sera of patients and controls against different gliadin preparations in ELISA tests. Activity is expressed as mean value for each group (n=25).

From these results we can conclude that common immunodeterminants (18) are probably expressed in the glyc-gli glycopeptide and in other gliadin preparations. These immunodeterminants bind antibodies present in the sera of patients with coeliac disease strongly and in a more specific way than antibodies present in the sera of control healthy subjects. Higher reactivity of IgG antibodies in comparison with IgA antibodies may be probably explained by the fact that most of our patients with coeliac disease were on a gluten-free diet.

Table I: Correlation Spearman's coefficients for each two of gliadin preparations in case of IgA antibodies in coeliacs (left) and IgG antibodies of controls (right). Insignificant values are marked by an asterisk.

Coeliacs								
IgA	α-gli	β-gli	γ-gli	ω-gli	A-gli	F-III	glyc-gli	
glyc-gli	0.843	0.911	0.724	0.803	0.953	0.752		glyc-gli
Frazer-F-III	0.576	0.643	0.717	0.459	0.655		0.574	Frazer-F-III
A-gliadin	0.870	0.950	0.666	0.839		*0.315	0.716	A-gliadin
ω-gliadin	0.807	0.920	0.662		0.464	0.639	0.681	ω-gliadin
γ-gliadin	0.613	0.735		*0.292	*0.182	*0.016	*0.108	γ-gliadin
β-gliadin	0.888		*0.104	0.872	0.536	0.630	0.765	β-gliadin
α-gliadin		0.594	*0.261	0.461	*0.303	0.571	0.532	α-gliadin
	α-gli	β-gli	γ-gli	ω-gli	A-gli	F-III	glyc-gli	IgG
								Controls

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