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GLUTEN, A LECTIN WITH OLIGOMANNOSYL SPECIFICITY AND THE CAUSATIVE AGENT OF GLUTEN-SENSITIVE ENTEROPATHY

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The pathogenesis of gluten-sensitive enteropathy or coeliac disease is as yet unknown. We can demonstrate by laser nephelometric measurements that gluten has lectin-like properties. Gluten binds 'high-mannose type' glycoproteins and the complex formation is inhibitable by mannan. As known for other lectins the reaction is absolutely Ca-dependent. Glycoproteins from the immature crypt cells from the small intestine are highly more reactive than glycoproteins from the mature villous zone. The possibility of a genetically determined deficiency of the growth-dependent N-acetyl-glucosaminyltransferase-1 as the pathogenic factor of the gluten-sensitive enteropathy is discussed.

Gluten-sensitive enteropathy (GSE) or coeliac disease is genetically determined, and has been shown by various investigators to be correlated with certain HLA-types (1,2). The true pathogenesis of this disease is still unknown. It has been suggested that gluten (gliadin) develops toxic properties in the intestinal tract, or that absorption in predisposed patients is followed by an immune response, with the synthesis of anti-gliadin antibodies. Recently, M. Weiser put forward the theory that gluten possesses lectin-like properties (3). By a mechanism that is well known for many other lectins (4), gluten would display membrane toxicity by binding to oligosaccharide units of glycoconjugates from the small intestine. The present investigation provides the first confirmation of this lectin theory, thus permitting further conclusions on the pathogenesis of GSE.

Abbreviations: GSE - gluten-sensitive enteropathy, GalNAc - N-acetyl galactosamine, GlcNAc - N-acetylglucosamine, Con A - Concanavalin A, RCA - ricinus communis agglutinin, SBA - soybean agglutinin, WGA - wheat germ agglutinin, α-2-HS-GP - α-2 Human Serum Glycoprotein.

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MATERIALS AND METHODS

Gluten was obtained from Sigma Chem. (St.Louis,MO,USA) and converted into water-soluble form by proteolytic cleavage with papain and pepsin (Sigma). Protein fraction III of gluten according to Frazer et. al. (5) was used in the present work, after first dialysing for 36 h against water. Protease activity was destroyed by heating the dissolved material for 45 min at 80° C.
This water-soluble gluten fraction was further characterized by chromatography on Sephadex, DEAE-Sephadex (Pharmacia Fine Chem., Uppsala,Sweden) and agarose-bound lectins (E.Y. Lab. Inc. (San Mateo, CA, USA). Polyacrylamide gel electrophoresis was performed as described earlier (6).
Lectin behaviour was demonstrated by agar diffusion and laser nephelometry (Behring Werke, Marburg, FRG), according to previously described methods (7,8).
All sugars used as inhibitory substances were obained from Sigma. The oligomannose fraction from the ovalbumin was prepared enzymatically by the method of (9).High purified glycoproteins were obtained from Behring. Fetuin and ovalbumin were from Sigma.
Intestinal brush border membranes from Sprague Dawley rats were isolated and fractionated into villous and cryptic zones according to M. Weiser (10).

RESULTS AND DISCUSSION

1. Characterization of the water-soluble gluten (fraction III).

In gel chromatography (Sephadex G-50) the gluten gave essentially a single peak with an apparent molecular weight of about 30,000 D. Under non-dissociating conditions, various gel-chromatographic methods showed no further resolution of this protein fraction, but in SDS-polyacrylamide gel electrophoresis it was separated into two fractions with molecular weigths of 31,500 and 15,500 D. Gluten ist not bound by Con A-Sepharose or by other carrier-bound lectins (WGA, RCA, SBA), so it may concluded that gluten is not a glycoprotein.
Using anti-gliadin antibodies from GSE patients, it was shown that our gluten preparation was the protein fraction responsible for toxicity.
The lectin-like behaviour of gluten (see below) is inhibited less than 10% by heating for 60 min at 80°C. On the other hand, incubation of the protein for more than 45 min at 100°C destroys more than 50% of the lectin properties. In order to exclude all protease or other enzyme activity from our experimental material, the dissolved gluten was therefore heated thoroughly for 45 min at 80°C before each experiment.

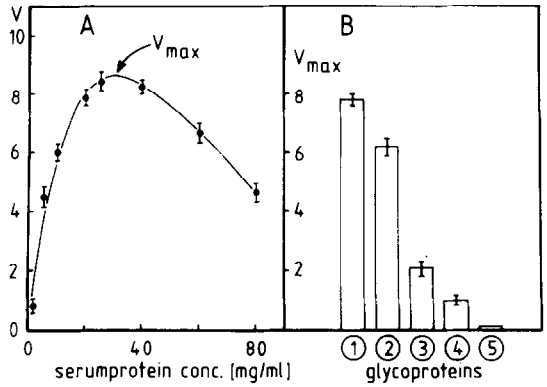

Fig. 1: Gluten reactivity towards human serum and glycoproteins. A: At a constant gluten concentration (2.5 mg/ml) and with a variable serum concentration (abscissa), high molecular weight complexes are formed, which can be quantified by laser nephelometry. (V=degree of turbidity or complex formation (volts)). For further details of methodology, see (7,8). B: When incubated with gluten (2.5 mg/ml) different glycoproteins show different values for maximal complex formation (V-max). 1=IgM, 2=ovalbumin, 3=IgA, 4=α-2-HS-GP, 5=transferrin. (n=5, $\bar{x}$ +/-s).

2. Demonstration of the lectin character of gluten.

Double diffusion of serum from normals persons against gluten in agar produces a precipitate within 24 h, similar to that observed in antigen-antibody reactions (Ouchterlony technique). Similarly, using a gluten containing agar, the so called Mancini technique gives a ring of precipitate dependent on the serum concentration (both not shown here).

An improved and more rapid quantification of this precipitate formation can be achieved with the aid of the laser nephelometry. Fig. 1 shows the concentration-dependent complex formation between serum from healthy individuals and gluten; as already shown for other lectins, this behaviour corresponds to the precipitation curve (Heidelberger curve) for the antigen-antibody interaction. The complex formation is pH-dependent, with an optimum at pH 8.0, and it is Ca^{2+}-dependent. All studies were therefore performed in 0.1 M Tris, pH 8.0, containing 10 mM $CaCl_2$.

Since deproteinized serum possesses no gluten reactivity, the binding behaviour of various defined serum proteins was tested. Many glycoproteins (transferrin, fetuin, haptoglobin, acidic α_1 GP, IgG, β_2GP, carboanhydrase, lactoferrin and others) show no kind of gluten reactivity. Other glycoproteins, however, show very pronounced complex formation with gluten; these include

IgM, ovalbumin, and in a reduced manner IgA and α_2-HS-GP (Fig.1B).

The only distinguishing feature between the gluten-reactive and non-reactive proteins is their differently structured carbohydrate moiety. All gluten non-reactive glycoproteins have the so called complex type glycosylation, whereas gluten-reactive glycoproteins possess the high-mannose type glycosylation. Non-glycosylated proteins, for example human or bovine serum albumin , do not react with gluten.

The gluten-binding properties of IgM or ovalbumin are abolished by chemical hydrolysis or by enzymic cleavage with endoglycosidase H. This strongly suggests that the defined oligosaccharide units of the glycoproteins are responsible for the binding of gluten. Confirmation is provided by inhibition experiments with mono- and oligosaccharides. Even in high concentrations (1M), galactose, glucose, fucose, GlcNAc, GalNAc and N-Acetylneuraminic acid possess no inhibitory activity. Neutral salts are equally inactive. Mannose, at a relatively high concentration of 0.3 M, shows 50% inhibition of gluten-ovalbumin complex formation. On the other hand, the high-mannose glycopeptide (protein content is less than 2%), mannan, shows 50% inhibition of gluten-ovalbumin complex formation at a concentration of 16 µM (0.73mg/ml).Competitive inhibition of gluten-glycoprotein interaction by mannan can also be verified by the Dixon plot, which is used in enzyme kinetic analysis (Fig. 2).

Preliminary studies show that a corresponding inhibition can also be achieved with the oligosaccharide units of ovalbumin. At the moment it is not known how many mannose residues are required for optimal reactivity with gluten.

3. Gluten reactivity towards intestinal glycoproteins.

In order to relate our findings to the pathogenesis of GSE, we also studied the gluten-binding ability of glycoproteins of the plasmamembrane of rat intestinal brush border.

Collectively, the glycoproteins isolated from the brush border exhibit an intermediate gluten reactivity, comparable to that of IgA. It is worth noting, however, that the strongest reaction with gluten, even in comparison with all other glycoproteins tested, is shown by the glycoproteins of the immature cryptic cells. Glycoproteins from the mature villous zones show only a minimal reaction (Fig. 3). Mannan is also a competitive inhibitor of these interactions.

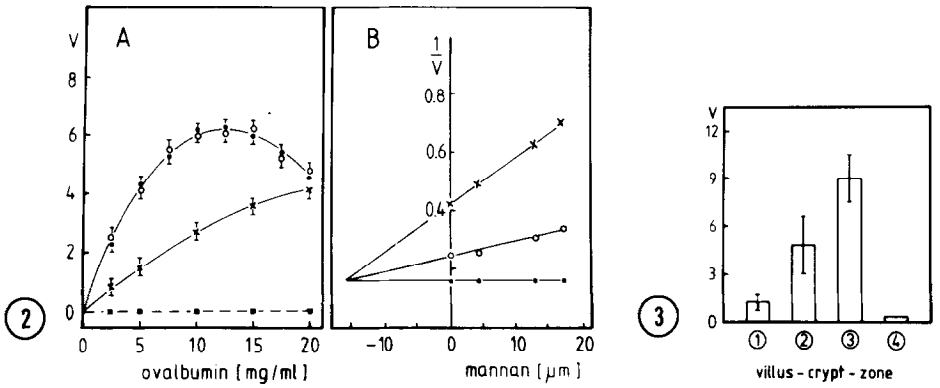

Fig. 2: Inhibition of the gluten-ovalbumin interaction.
A: Concentration-dependent complex formation between ovalbumin and gluten (2.5 mg/ml) (●). Addition of glucose (0.3 M) does not change the reaction (○). The same is true for other monosaccharides that are present as components of complex-type glycoproteins. An exception is mannose, which causes a concentration-dependent decrease in gluten-ovalbumin binding (x=0.3 M mannose). Mannan inhibits the reaction completely at a concentration of 0.1 M (■).
B: To establish the type of inhibition exerted by mannan on the gluten-ovalbumin complex formation, and to determine the inhibitor constant, the interaction of gluten, ovalbumin and mannan can be presented as a Dixon plot. Various concentrations of ovalbumin (●=15, ○=5, x=2.5 mg/ml) were added to the gluten solution (2.5 mg/ml). To these test mixtures, increasing quantities of mannan were added as inhibitor (abscissa). According to the position of the intercept of the lines, mannan is a competitive inhibitor of the gluten-glycoprotein interaction. Projection of the intercept to the negative part of the abscissa permits the determination of the inhibitor constant for mannan.

Fig. 3: Ability of gluten to bind to glycoproteins of intestinal brush border.
Glycoproteins from the mature villous zone of the small intestine of rats (Sprague Dawley, 200 g) show only a very slight ability to bind gluten (1). This binding is higher in the intermediate zone between villous and crypt cells (2) and becomes maximal in the immature cryptic cells (3). Here also, mannan (5 mg/ml) completely inhibits complex formation between gluten and cryptic cell glycoproteins (4). In each experiment the gluten concentration was 2.5 mg/ml, and the glycoprotein concentration was 1.0 mg/ml. These results represent the mean values (x +/-s) from 5 different small intestine preparations.

On the basis of these findings it seems very probable that gluten, by virtue of its lectin properties, binds to oligomannose residues of glycoconjugates of the intestinal tract. In patients with GSE, a higher proportion of oligomannosyl-glycoproteins in the brush border membrane could be responsible for the increased gluten binding. Thus, it is known that the activity of GlcNAc-Transferase-1 depends on the stage of cell maturation (11,12).

This enzyme is necessary for the synthesis of the oligosaccharide moiety of complex-type glycoproteins. Cell lines deficient in GlcNAc-Transferase-1 no longer synthesise complex-type glycoproteins, but produce glycoproteins with terminal oligomannosyl units similar to the high-mannose-type glycoproteins (13). We are currently investigating patients with GSE for this type of enzyme deficiency. This would not only explain the pathogenesis of this disease; it would also offer the possibility of important new therapeutic measures based on the competitive inhibition of the lectin-like properties of gluten.

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