COMMUNICATION TO THE EDITOR

Absence of Carbohydrate in Celiac-Toxic A-Gliadin[1]

Cereal Chemistry 53(4): 612-614

TO THE EDITOR:

The toxicity of gliadin proteins in celiac disease has usually been attributed to peptides derived from the gliadins as a consequence of proteolytic degradation

[1]Reference to a company or product name does not imply approval or recommendation of the product by the U.S. Department of Agriculture to the exclusion of others that may be desirable.

during the digestive process (1). It has been suggested recently that carbohydrate bound chemically to gliadin molecules plays a critical role in determining the toxicity of these proteins (2). Because the toxicity of A-gliadin (3) (aggregable α-gliadin (4)) has been established (5–7), we have analyzed this protein fraction for carbohydrate in order to test the possibility that its toxicity stems from chemically bound carbohydrate.

The A-gliadin was prepared as described previously (4), with two exceptions: a Bio-Gel P-60 (Bio-Rad, U.S.A.) column was substituted for the Sephadex G-100 column in order to avoid any possible carbohydrate contamination from the Sephadex, and 0.006*M* ammonium formate (adjusted to pH 3.0) was used as eluting solvent instead of 0.001*M* hydrochloric acid. Care was taken to avoid contamination of the sample with dust during preparation and freeze-drying.

Analysis of duplicate 14-mg samples of A-gliadin for total carbohydrate, according to the method of Dubois *et al.* (8), yielded a carbohydrate content equivalent to 0.07 mol glucost/mol A-gliadin (mol wt 36,000) (3); as little as 0.02 mol/mol would have been detectable under the conditions we used. In addition, the A-gliadin was analyzed for amino sugars, which would not have been detected by the method of Dubois *et al.* (8), by the method of Mes and Kamm (9). The protein was hydrolyzed in 2*N* hydrochloric acid for 16 hr at 105° C and the hydrochloric acid removed by evacuating the sample tubes and flushing them with nitrogen. A sample equivalent to 1.6 mg of protein was used for paper chromatography (9) (Whatman No. 1 paper; n-butanol-pyridine-water, 10:3:3 v/v/v; silver nitrate-alkaline visualization). N-Acetylglucosamine and N-acetylgalactosamine served as standards. No amino sugars or other sugars were detected, even though 0.1 mol sugar/mol A-gliadin should have been detected under the conditions of the analysis.

The amount of carbohydrate in our A-gliadin preparation was equivalent to only one molecule of monosaccharide for every 10 molecules of protein. This small amount of carbohydrate probably represents impurities in the preparation; we conclude that A-gliadin is not a glycoprotein. We cannot rule out the possibility that the small amount of carbohydrate might result from contamination of our preparation with a small amount of some glycoprotein, nor can we rule out the possibility that such a contaminant might be highly toxic in celiac disease and responsible for the toxicity of A-gliadin preparations. This latter possibility seems most unlikely to us, however, and we conclude that the toxicity of A-gliadin preparations is not dependent upon chemically bound carbohydrate.

JOHN E. BERNARDIN
ROBIN M. SAUNDERS
DONALD D. KASARDA
Western Regional Research Laboratory
Agricultural Research Service
U.S. Department of Agriculture
Berkeley, CA 94710

Literature Cited

1. KASARDA, D. D. Celiac disease: Malabsorption of nutrients induced by a toxic factor in gluten, p. 565. In: Protein nutritional quality of foods and feeds, Part II, ed. by M. Friedman. Marcel Dekker: New York (1975).

2. PHELAN, J. J., McCARTHY, C. F., STEVENS, F. M., McNICHOLL, B., and FOTTRELL, P. F. The nature of gliadin toxicity in coeliac disease: A new concept, p. 60. In: Coeliac disease. Proc. 2nd Int. Symp., ed. by W. Th. J. M. Hekkens and A. S. Pena. H. E. Stenfert Kroese B.V.: Leiden (1974).
3. PLATT, S. G., KASARDA, D. D., and QUALSET, C. O. Varietal relationships of the α-gliadin proteins in wheat. J. Sci. Food Agr. 25: 1555 (1974).
4. BERNARDIN, J. E., KASARDA, D. D., and MECHAM, D. K. Preparation and characterization of α-gliadin. J. Biol. Chem. 242: 445 (1967).
5. HEKKENS, W. Th. J. M., HAEX, A. J. Ch., and WILLIGHAGEN, R. J. G. Some aspects of gliadin fractionation and testing by a histochemical method, p. 11. In: Coeliac disease. Proc. Int. Symp., ed. by C. C. Booth and R. H. Dowling. Churchill Livingstone: London (1970).
6. HEKKENS, W. Th. J. M., VAN den AARSEN, C. J., GILLIAMS, J. P., LEMS-VAN KAN, Ph., and BOUMA-FRÖLICH, G. α-Gliadin structure and degradation, p. 39. In: Coeliac disease. Proc. 2nd Int. Symp., ed. by W. Th. J. M. Hekkens and A. S. Pena. H. E. Stenfert Kroese B.V.: Leiden (1974).
7. FALCHUK, Z. M., GEBHARD, R. L., SESSOMS, C., and STROBER, W. An in vitro model of gluten-sensitive enteropathy: Effect of gliadin on intestinal epithelial cells of patients with gluten-sensitive enteropathy in organ culture. J. Clin. Invest. 53: 487 (1974).
8. DUBOIS, M., GILLES, K. A., REBERS, P. A., and SMITH, F. Colorimetric method for determination of sugars and related substances. Anal. Chem. 28: 350 (1956).
9. MES, J., and KAMM, L. The relative sensitivity of various reagents for the detection and differentiation of sugars and sugar derivatives in glycoproteins. J. Chromatogr. 38: 120 (1968).