

The Amino Acid Composition of D Hordein

To the Editor:

I write to comment on the recent paper by Blake et al (1984) on the purification and characterization of microgram quantities of D hordein from three cultivars of barley. The most striking feature of the amino acid compositions reported for these preparations was the high and variable content of glycine (14.6–26.9 mol %). This high glycine content is known to be characteristic of D hordein and the homologous high molecular weight (HMW) glutenin subunits of wheat and rye (Huebner et al 1974, Khan and Bushuk 1979, Field et al 1982, Shewry et al 1984). However, the range of variation is, in our experience, unusually high for D hordein. We have purified milligram amounts of D hordein from two cultivars of barley using conventional procedures of protein chemistry. One fraction, from the cultivar Sundance, contained 13.6 mol % glycine (Field et al 1982). The second fraction was from the mutant line Risø 1508, which has the same structural allele at the *Hor 3* locus (which encodes D hordein [Blake et al 1982, Shewry et al 1983]) as Bomi. Our analysis showed 15.7 mol % glycine (Kreis et al 1984), compared with 26.9 mol % reported for D hordein of Bomi by Blake et al (1984). This discrepancy is clearly outside the normal range of experimental error. The answer may lie in the methods used to prepare the proteins. Blake et al (1984) used preparative SDS-PAGE, based on the system of Laemmli (1970). Small amounts of glycine originating from the Tris/glycine running buffer used in this system could result in appreciable contamination of microgram quantities of purified proteins.

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LITERATURE CITED

- BLAKE, T. K., ULLRICH, S. E., and NILAN, R. A. 1982. Mapping of the *Hor 3* locus encoding D hordein in barley. *Theor. Appl. Genet.* 63:367.
- BLAKE, T. K., ULLRICH, S. E., and NILAN, R. A. 1984. Purification and characterization of barley D hordein. *Cereal Chem.* 61:120.
- FIELD, J. M., SHEWRY, P. R., MIFLIN, B. J., and MARCH, J. F. 1982. The purification and characterization of homologous high molecular weight storage proteins from the grain of wheat, rye and barley. *Theor. Appl. Genet.* 62:329.
- HUEBNER, F. R., DONALDSON, G. L., and WALL, J. S. 1974. Wheat gluten subunits. II. Compositional differences. *Cereal Chem.* 51:240.
- KHAN, K., and BUSHUK, W. 1979. Studies of glutenin. XIII. Gel filtration, isoelectric focusing, and amino acid composition studies. *Cereal Chem.* 56:505.
- KREIS, M., SHEWRY, P. R., FORDE, B. G., RAHMAN, S., BAHRAMIAN, M. B., and MIFLIN, B. J. 1984. Molecular analysis of the effects of the *lys 3a* gene on the expression of *Hor* loci in developing endosperms of barley (*Hordeum vulgare* L.). *Biochem. Genet.* 22:231.
- LAEMMLI, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 227:680.
- SHEWRY, P. R., FIELD, J. M., FAULKS, A. J., PARMAR, S., MIFLIN, B. J., DIETLER, M. D., LEW, E. J.-L., and KASARDA, D. D. 1984. The purification and N-terminal amino acid sequence analysis of the high molecular weight gluten polypeptides of wheat. *Biochim. Biophys. Acta* 788:23.
- SHEWRY, P. R., FINCH, R. A., PARMAR, S., FRANKLIN, J., and MIFLIN, B. J. 1983. Chromosomal location of *Hor 3*, a new locus governing storage proteins in barley. *Heredity* 50:179.

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Dr. Shewry's concern over glycine contamination from the Laemmli buffer system that we used as a preparative step is reasonable, and we had the same concern. To minimize glycine-based contamination, we electroeluted the protein in a nonglycine-containing buffer overnight, prior to exhaustive dialysis. We were working with extremely small samples and consequently any contamination that did remain would provide larger errors than if we had been able to scale up to milligram quantities.

The major points of the paper were 1) to demonstrate that D hordein and HMW glutenin have some significant points in common (eg, their fragmentation with *Staphylococcus aureus* V-8 protease, although not identical, shows similarities); 2) to demonstrate that useful information could be gathered from small samples; and 3) to provide a method by which a larger and better-funded project could purify enough peptides of a useful size distribution to perform a useful sequence analysis. I believe protein sequence analyses are a crucial complement to nucleic acid sequence analysis in distinguishing between real genes and pseudogenes.

The real question raised by Dr. Shewry revolves around whether the variability that we observed between samples is real or an artifact of our preparative methods. The work of Khan and Bushuk (1979) indicates that HMW glutenins in wheat may contain up to 20.4% glycine. My observation of 20.6% is quite close to their estimates, while Dr. Shewry's (Field et al 1982) estimate of 14.6% is substantially lower. The variability between barley varieties that we observed (14–26% glycine) is indeed large but, in my opinion, suggests that there may be significant differences in D hordein composition between barley genotypes that are of widely divergent origin, as there are apparently large differences among analogous proteins in wheat.

While Bomi and Risø 1508 do presumably carry the same D hordein structural genes, we know little or nothing about the developmental regulation of these genes. Bomi and Risø 1508 also carry identical B and C hordein structural genes, but in Risø 1508 the genes show dramatically altered regulation, which does indeed evidence itself in different hordein compositions between these genotypes. One needs to be careful in distinguishing between structure and function.

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LITERATURE CITED

- FIELD, J. M., SHEWRY, P. R., MIFLIN, B. J., and MARCH, J. F. 1982. The purification and characterization of homologous high molecular weight storage proteins from the grain of wheat, rye and barley. *Theor. Appl. Genet.* 62:329.
- KHAN, K., and BUSHUK, W. 1979. Studies of glutenin. XIII. Gel filtration, isoelectric focusing, and amino acid composition studies. *Cereal Chem.* 56:505.