Biochemical Genetics, Vol. 29, Nos. 3/4, 1991

α-Type Prolamins Are Encoded by Genes on Chromosomes 4Ha and 6Ha of *Haynaldia villosa* Schur (syn. *Dasypyrum villosum* L.)

Peter R. Shewry,[1] Paolo A. Sabelli,[1] Saroj Parmar,[2] and Domenico Lafiandra[3]

Received 16 Jan. 1991—Final 16 Jan. 1991

Because of their high degree of polymorphism and structural diversity, the prolamin storage proteins of grasses have proved to be valuable markers in taxonomic studies. We have used this approach to suggest that the wild grass *Haynaldia villosa* is more closely related to cultivated wheat than to rye (Shewry *et al.,* 1987), a result which conflicts with detailed numerical studies (Baum, 1977, 1978). In particular, seeds of *H. villosa* contain α-type gliadins, a group of prolamins previously reported only in cultivated wheat and closely related species of *Triticum, Aegilops,* and *Agropyron* (*Elytrigia*) (Autran *et al.,* 1979; Dvorak *et al.,* 1986). Two-dimensional electrophoretic analyses of addition lines of *H. villosa* chromosomes in bread wheat (cv. Chinese Spring) showed the presence of prolamins encoded by chromosomes 1Ha and 6Ha, the latter being presumed to encode the α-type gliadins (by homology with the *Gli-2* loci on the group 6 chromosomes of bread wheat). However, analyses of the same series of addition lines by Montebove *et al.* (1987) failed to confirm the presence of gliadin genes on chromosome 6Ha but did show putative α-type gliadins encoded by 4Ha. Furthermore, Blanco *et al.* (1991) showed α-type gliadins encoded by chromosomes 4Ha *and* 6Ha using a separate series of lines added into pasta wheat.

The different results reported by Shewry *et al.* (1987) and Montebove *et al.* (1987) may relate to the electrophoretic systems which were used: two-dimensional (2-D) isoelectric focusing/sodium dodecyl sulfate–poly-

[1] Department of Agricultural Sciences, University of Bristol, AFRC Institute of Arable Crops Research, Long Ashton Research Station, Bristol, BS18 9AF, U.K.
[2] AFRC Institute of Arable Crops Research, Rothamsted Experimental Station, Harpenden, Herts, AL5 2JQ, U.K.
[3] Department of Agrobiology and Agrochemistry, University of Tuscia, via de Lellis, 01100 Tuscia, Italy.

0006-2928/91/0400-0207$06.50/0

acrylamide gel electrophoresis (SDS-PAGE) and 1-D acid PAGE, respectively. We have therefore separated the alcohol-soluble (1.5 M aq. dimethylformamide) proteins from Chinese Spring wheat and the 4Ha and 6Ha addition lines using an alternative high-resolution 2-D system, combining separations at *p*H 3.2 and 9.2. This clearly demonstrated single additional proteins with mobilities similar to those of α-type gliadins in the chromosome 4Ha and 6Ha addition lines (arrowed in Figs. 1b and c). In order to confirm their identities the proteins were transferred from 2-D gels onto immobilon membrane and briefly stained with Coomassie BBR 250. Spots from several transfers were cut out, bulked, and submitted to N-terminal sequencing using an Applied Biosystems Model 477A Pulsed Liquid Phase Sequencer. This procedure was initially tested on an authentic α-type gliadin from Chinese Spring, indicated in Fig. 1a. The sequence determined for the first 10 residues was identical to that established for α-type gliadins on the basis of direct protein sequencing (Bietz *et al.,* 1977) and the analyses of cDNAs and genes (see Kreis *et al.,* 1985) (Table I). An identical sequence was determined for the gliadin encoded by chromosome 6Ha, confirming that it is a typical α-type gliadin. In contrast the gliadin encoded by chromosome 4Ha had a variant type of sequence, with an additional leucine residue (position −1 in Table I) and leucine instead of valine at position 1. This was similar to the N-terminal sequence reported previously for an α-gliadin mixture purified from *H. villosa* seeds (Shewry *et al.,* 1987). However, in this case a solid phase sequencing system was used, and the N-terminal residue was tentatively (and probably erroneously, in light of the present results) identified as glutamine.

The α-type gliadins encoded by chromosomes 4Ha and 6Ha of *H. villosa* are quantitatively minor components, most of the prolamins being encoded by genes on chromosome 1Ha (Shewry *et al.,* 1987). This is similar to the situation in other members of the tribe Triticeae, and it is considered that all prolamin genes were originally present on chromosomes homologous with the group 1 chromosomes of wheat but, in some cases, have been translocated to other chromosomes. Thus prolamin genes are present only on chromosome 5 (1H) of barley, on chromosomes 1E and 6E of *Agropyron* (*Elytrigia*) (Dvorak *et al.,* 1986), on chromosomes 1R and 2R of cultivated rye (Shewry *et al.,* 1986), and on the group 1 and 6 chromosomes of wheat (Payne *et al.,* 1984). The α-gliadin genes on chromosomes 6A, 6B, and 6D of wheat and on 6Ha and 6E are considered to have evolved from genes encoding ancestral α-type gliadins, which have been transferred from the group 1 chromosomes. The results reported here indicate that a second translocation of α-gliadin genes has occurred in the ancestor of *H. villosa,* from chromosome 6Ha to 4Ha.

α-Gliadin genes and proteins are not apparently present in barley, and

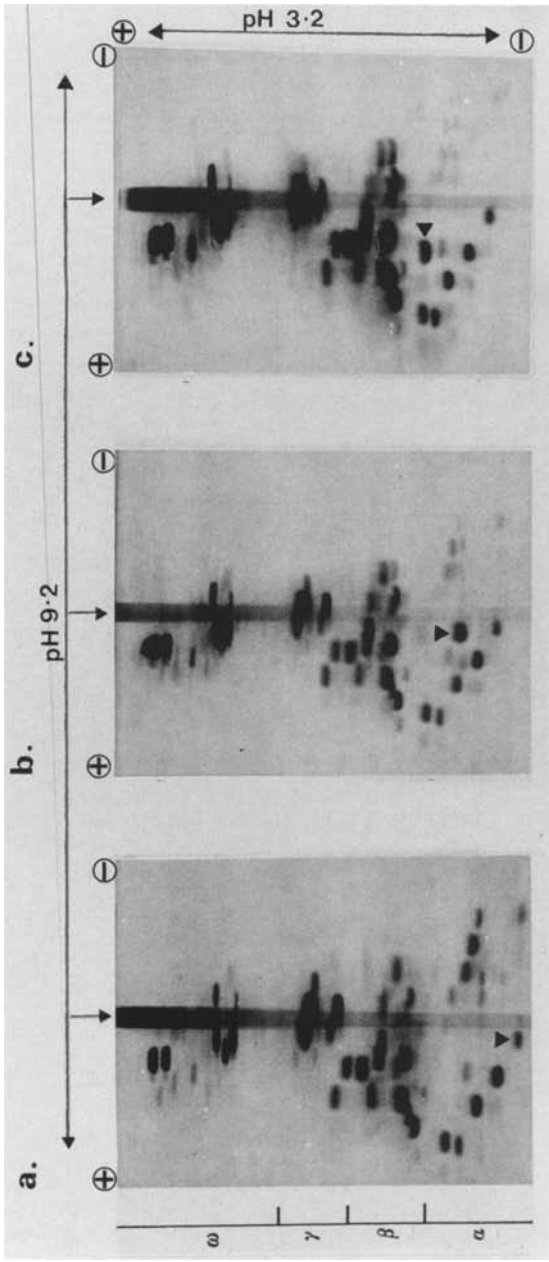

Fig. 1. 2-D (*p*H 3.2 followed by *p*H 9.2) electrophoresis of 1.5 M dimethylformamide-soluble proteins from wheat cv. Chinese Spring (a) and addition lines of *H. villosa* chromosomes 4Ha (b) and 6Ha (c) into Chinese Spring. The α^1-gliadin and the α-type gliadins encoded by chromosomes 4Ha and 6Ha are indicated by arrowheads. The methods used were as described by Lafiandra *et al.* (1985). α, β, γ, and ω indicate the groups of gliadins defined on electrophoretic mobility at low *p*H.

Table I. Comparison of the N-Terminal Sequences Determined for Wheat and *H. villosa* Proteins by Microsequencing with Those Determined Previously by Conventional Sequencing of Purified Proteins[a]

	−1	1				5					10
Microsequencing											
Wheat α[1]-gliadin		V	(R)	V	P	V	P	Q	L	Q	P
H. villosa											
4Ha gliadin	I	L	(R)	V	P	V	P	Q	L	Q	
6Ha gliadin		V	(R)	V	P	V	P	Q	L	Q	P
Conventional sequencing											
Wheat α-type gliadins[b]		V	R	V	P	V	P	Q	L	Q	P
H. villosa α-gliadin[c]	(Q)	L	R	V	P	V	P	Q	L	Q	S

[a]Standard single-letter abbreviations are used: I, isoleucine; L, leucine; P, proline; Q, glutamine; R, arginine; V, valine. Yields of residues varied between about 4 and 20 pmol, except for arginine, which was recorded only in trace amounts.
[b]The wheat α-type gliadin sequence is typical of all α-type (i.e., α and β) gliadins (see Bietz *et al.*, 1977).
[c]The *H. villosa* α-gliadin was purified by Shewry *et al.* (1987).

it must be assumed that the initial translocation occurred after the divergence of the ancestors of barley and wheat. Despite this fairly recent origin the α-gliadins are one of the most divergent groups, with little sequence homology with other gliadins. We have speculated that this apparently rapid divergence may be related to the translocation event, either as a direct result or from accelerated divergence due to spread of mutations by gene conversion events (Kreis and Shewry, 1989). Although there is no direct evidence to support this hypothesis, it is of interest that the α-gliadin gene(s) on chromosome 4Ha differs from those on chromosome 6Ha in the N-terminal sequences of the encoded proteins.

ACKNOWLEDGMENTS

The N-terminal amino acid sequences were determined at the University of Bristol Molecular Recognition Centre. We are grateful to Professor E.R. Sears for supplying the addition lines.

REFERENCES

Autran, J.-C., Lew, E. J.-L., Nimmo, C. C., and Kasarda, D. D. (1979). N-Terminal amino acid sequencing of prolamins from wheat and related species. *Nature (London)* **282:**527.

Baum, B. R. (1977). Taxonomy of the tribe Triticeae (*Poaceae*) using various numerical techniques I. *Can. J. Bot.* **55:**1712.

Baum, B. R. (1978). Genetic relationships in Triticeae based on computations of Jardine & Sibson Bk clusters. *Can. J. Bot.* **56:**2948.

Bietz, J. A., Huebner, F. R., Sandersen, J. E., and Wall, J. S. (1977). Wheat gliadin homology revealed through N-terminal amino acid sequence analysis. *Cereal Chem.* **54:**1070.

Blanco, A., Resta, P., Simeone, R., Parmar, S., Shewry, P. R., Sabelli, P., and Lafiandra, D. (1991). Chromosomal location of seed storage protein genes in the genome of *Dasypyrum villosum* (L) Candargy *Theor. Appl. Genet.* (in press).

Dvorak, J., Kasarda, D. D., Dietler, M. D., Lew, E. J.-L., Anderson, O. W., Litts, J. C., and Shewry, P. R. (1986). Chromosomal location of seed storage protein genes in the genome of *Elytrigia elongata. Can. J. Genet. Cytol.* **28:**818.

Kreis, M., and Shewry, P. R. (1989). Unusual features of cereal seed protein structure and evolution. *BioEssays* **10:**201.

Kreis, M., Shewry, P. R., Forde, B. G., Forde, J., and Miflin, B. J. (1985). Structure and evolution of seed storage proteins and their genes, with particular reference to those of wheat, barley and rye. In Miflin, B. J. (ed.), *Oxford Surveys of Plant Cell and Molecular Biology, Vol 2,* Oxford, London, pp. 253–317.

Lafiandra, D., and Kasarda, D. D. (1985). One- and two-dimensional (2-pH) polyacrylamide gel electrophoresis in a single gel: Separation of wheat protein. *Cereal Chem.* **62:**314.

Montebove, L., De Pace, C., Jan, C. C., Scarascia Mugnozza, G. T., and Qualset, C. O. (1987). Chromosomal location of isozyme and seed storage protein genes in *Dasypyrum villosum* (L) Candargy. *Theor. Appl. Genet.* **73:**836.

Payne, P. I., Holt, L. M., Jackson, E. A., and Law, C. N. (1984). Wheat storage proteins: Their genetics and their potential for manipulation by plant breeding. *Phil. Trans. R. Soc. Lond. B* **304:**373.

Shewry, P. R., Parmar, S., Fulrath, N., Kasarda, D. D., and Miller, T. E. (1986). Chromosomal locations of the structural genes for secalins in wild perennial rye (*Secale montanum* Guss) and cultivated rye (*S. cereale* L.) determined by two-dimensional electrophoresis. *Can. J. Genet. Cytol.* **28:**76.

Shewry, P. R., Parmar, S., and Pappin, D. J. C. (1987). Characterization and genetic control of the prolamins of *Haynaldia villosa:* relationship to cultivated species of the Triticeae (rye, wheat and barley). *Biochem. Genet.* **25:**309.