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Chromosomal control of the aminopeptidases of wheat and its close relatives

R. M. D. Koebner and P. K. Martin

I.P.S.R. Cambridge Laboratory, Maris Lane, Trumpington, Cambridge CB2 2JB, UK

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Summary. Isoelectric focussing was used to separate the isozymes of aminopeptidase of wheat and its relatives. Three distinct homoeoallelic sets of genes have been shown to be present. AMP-1, controlled by genes on the long arms of group 6, has previously been described, but two new systems, AMP-2 (group 4) and AMP-3 (group 7) are described here. The three systems are distinguished by their electrophoretic characteristics, by their genetic control and by their substrate specificity. Intervarietal, interspecific and intergeneric polymorphism has been observed at most of the loci. A further set of isozymes, AMP-4, was detected but the chromosomal control of these could not be determined.

Key words: Aminopeptidase – Wheat – Isoelectric focussing – Isozymes

Introduction

Aminopeptidases are a group of enzymes common to all higher plants. They are capable of the hydrolysis of peptide bonds between the α-carboxyl group of various amino-acids and the amino group of certain aromatic amines, such as βNA (β-Naphthylamide) (Mikola and Mikola 1986). There appear to be up to five distinct enzymes in most plant species, and these can be differentiated according to the particular amino acids favoured as substrate. In maize and rice, four aminopeptidases have been genetically defined (Vodkin and Scandalios 1979; Wu et al. 1988) and in barley, three have been biochemically differentiated (Mikola and Kohlemainen 1972). In wheat, however, only one aminopeptidase, AMP-1 (also referred to as leucine aminopeptidase or LAP) (Schmidt and Seliger 1982), has been genetically defined (Hart 1973), although multiple forms are present in wheat leaves (Waters and Dalling 1979). Kruger and Preston (1978) investigated the specificity of the βNAs of leucine (AMP-1), methionine, phenylalanine and tyrosine, but could only obtain AMP-1 activity. In the present work, it is demonstrated that wheat carries at least three distinct aminopeptidases, based on substrate specificity, electrophoretic characteristics and genetic control, and that at least one of these hereto undescribed isozymes shows a significant level of both intra- and interspecific and intergeneric polymorphism.

Materials and methods

Plant materials. The following genotypes and genetic stocks were analysed: bread wheat, *Triticum aestivum*, cv Chinese Spring (CS) and its nullisomic-tetrasomic (Sears 1966) and ditelosomic (Sears and Sears 1978) aneuploids; intervarietal chromosome substitutions involving CS as recipient parent with donor parents cvs Hope and Timstein (Sears et al. 1957), and Synthetic and *T. spelta* (I.P.S.R. Index No. 1220017) (Law and Worland, unpublished results) – synthetic is an artificial amphiploid from the cross *T. dicoccum* × *Aegilops squarrosa* (McFadden and Sears 1946); a set of F_6 single-seed descent lines obtained from the cross Sportsman × Highbury (J. W. Snape, I.P.S.R. Cambridge Laboratory); alien additions of the chromosomes of *Secale cereale* cv Imperial (Driscol and Sears 1971), *Hordeum vulgare* cv Betzes (Islam et al. 1981). *H. chilense* (Miller et al. 1982), *Haynaldia villosa* (E. R. Sears, unpublished results) and *Agropyron elongatum* (Dvorak and Knott 1974) to CS, and of *Ag. intermedium* to wheat cv Vilmorin 27 (Cauderon et al. 1978); the substitution line, having chromosome 4B replaced by *Aegilops sharonensis* chromosome $4S^1$ (Chapman and Miller 1979); the amphiploid CS × *Ag. junceum* and the putative chromosome 4J addition line (Forster and Miller 1985, unpublished results). Note that the new designations for chromosomes 4A and 4B, as agreed at the 7th International Wheat Genetics Symposium, Cambridge 1988, and published in the Proceedings (Miller and Koebner 1988), are used in this paper.

Electrophoresis. Crude aqueous extracts for electrophoresis were prepared by incubating crushed mature embryo or endosperm tissue from a single seed in 40 µl of distilled water or of 10 m*M* DTT (dithiothreitol) in distilled water at room temperature for 1–2 h. Prior to sample loading, the extracts were briefly centrifuged and about 15 µl of supernatant was loaded on the gel, either directly into wells punched into a silicon rubber strip placed parallel to and 10 mm from the cathode electrode, or indirectly, at a similar distance from the cathode, via a 5 × 10 mm paper application wick (Pharmacia). The polyacrylamide IEF (isoelectric focussing) gels contained 2% w/v ampholyte [a mixture of Pharmalyte 4.2–4.9 (Pharmacia) and Isolyte 3–5 (Isolabs) in the ratio 3:1], were 0.25 mm thick and normally 120 mm wide, and were run at 1 W/cm length, with voltage limited to 3 kV. Samples were loaded after a 500 V h prefocus, and the run was terminated after 4 kV h. In some cases 200-mm wide gels were run; for these, the sample origin was 50 mm from the cathode, the prefocus 1 kV h and the full run 12 kV h at 1 W/cm width. The catholyte was 0.5 *M* HEPES [N-(2-hydroxyethyl) piperazine-N'-(2-ethanesulfonic acid)], and the anolyte 1 *M* H_3PO_4. Gel staining followed Koebner et al. (1988), with the substitution of 0.5 mg/ml of the appropriate aminoacyl βNA for BANA (Nα-benzoyl-DL-arginine-β-naphthylamide). The following aminoacyl groups gave focussable activity on these gels: leucine, lysine, arginine, methionine, tryptophan and phenylalanine.

Results

Four distinct sets of isozymes (AMP-1, -2, -3 and -4) could be identified. These could be distinguished by specificity of substrate, pI (isoelectric point) and chromosomal control.

Chromosomal control of and polymorphism for Amp-1

AMP-1 was active against all aminoacyl groups assayed, but leucine was the substrate of choice, as AMP-2, -3 and -4 were not able to as effectively cleave this peptide bond in extracts of mature grain. Activity was most easily detectable in embryo extracts, but was also present in endosperm (particularly when extracted in the presence of 10 m*M* DTT) and young leaf. In young leaves, a low level of AMP-2 activity was also present. The pattern of CS showed two major isozymes (focussing between pH 4.3 and 4.35) and a third, faint isozyme of lower pI (Fig. 1 A, tracks a and i). The isozyme of highest pI is absent in stocks lacking chromosome 6B, but present in ditelosomic 6BS (DT6BS) (tracks d and e, respectively), while the other major isozyme is absent in stocks deficient for chromosome 6D or its long arm, but is present in DT6DS (tracks f–h). The third faint band is possibly reduced in intensity in the AMP-1 pattern of nullisomic 6A tetrasomic 6D, but is indistinguishable from the euploid intensity in extracts of ditelosomic 6AS (tracks b and c, respectively) and the remaining nullisomic-tetrasomics of CS (not shown). These results are fully consistent with the presence of a homoeoallelic series of structural genes, *Amp-1*, on the short arms of the group 6 chromosomes of wheat (Hart 1973).

In a limited survey of wheat genotypes, only two polymorphisms were uncovered at *Amp-1*. One of these occurs in the *T. spelta*, and analysis of the CS (*T. spelta*) substitution lines shows this to be due to a variant allele at *Amp-B1*. Vapa and Hart (1987) have reported that cv Iskra carries a variant allele at *Amp-B1* (*Amp-B1b*); however, our system is unable to differentiate the pattern of this variety from that of CS. The product of the *T. spelta* allele *Amp-B1c* focusses at a more acidic pH to that of the CS *Amp-B1a* allele, as demonstrated by the observation that the variant isozyme is produced in CS (*T. spelta* 6B), while the other substitutions of *T. spelta* chromosomes into CS produce only CS isozymes (Fig. 1 B, tracks e–i). Similarly, Synthetic can be shown to carry the *Amp-D1b* allele, whose product focusses at a more basic pH than that of the CS *Amp-D1a* allele (tracks a–e).

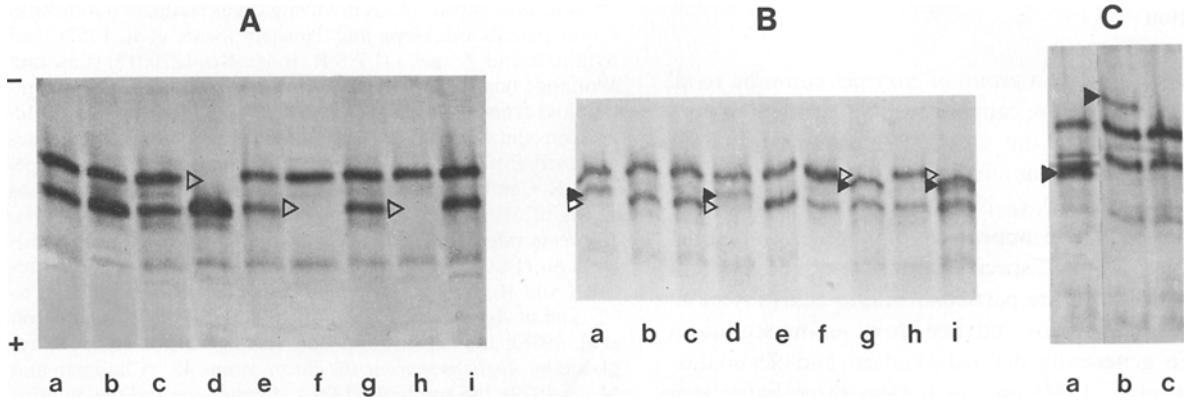

Fig. 1 A–C. AMP-1 patterns visualised by IEF. *Closed arrowheads* mark bands not present in Chinese Spring (CS), *open arrowheads* the absence of CS bands. **A** Aneuploid analysis. *a* – CS; *b* – Nullisomic 6A tetrasomic 6B (N6AT6B); *c* – Ditelosomic (DT)6AS; *d* – N6BT6A; *e* – DT6BL; *f* – N6DT6A; *g* – DT6DL; *h* – DT6DS; *i* – CS. **B** Chromosomal control of AMP-1. *a* – Synthetic; *b* – CS (Synthetic 6A); *c* – CS (Synthetic 6B); *d* – CS (Synthetic 6D); *e* – CS; *f* – CS (*T. spelta* 6B); *g* – CS (*T. spelta* 6B); *h* – CS (*T. spelta* 6D); *i* – *T. spelta*. **C** Alien homoeoloci of *Amp-1*. *a* – Vilmorin 27 + 6Ag^i; *b* – CS + 6H; *c* – CS

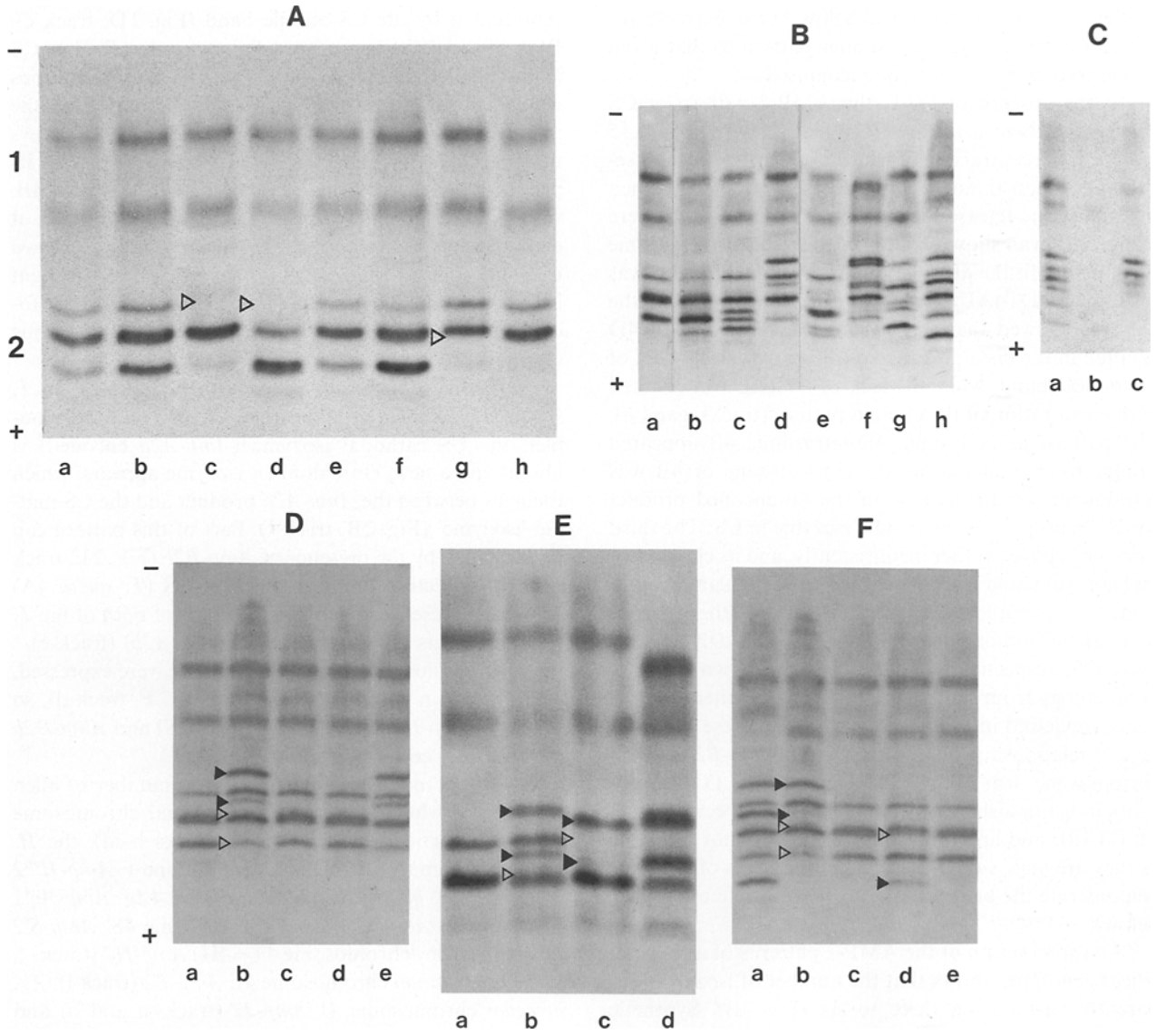

Fig. 2 A–F. AMP-2 patterns visualised by IEF. *Closed arrowheads* mark bands not present in Chinese Spring (CS), *open arrowheads* the absence of CS bands. 1, 2 denote the activity of AMP-1 and AMP-2, respectively. **A** Aneuploid analysis. *a* – CS; *b* – Ditelosomic (DT)4BS; *c* – Nullisomic 4A tetrasomic 4B(N4AT4B); *d* – N4AT4D; *e* – DT4AL; *f* – N4DT4B; *g* – N4DT4A; *h* – DT4DL. **B** Intervarietal variation. *a* – CS; *b* – Bersée; *c* – Hope; *d* – Timstein; *e* – Cheyenne; *f* – *T. spelta*; *g* – *T. macha*; *h* – Synthetic. **C** Synthetic and its progenitors. *a* – Synthetic; *b* – *Ae. squarrosa*; *c* – *T. dicoccum*. **D** Chromosomal control of AMP-2. *a* – CS; *b* – CS (Timstein 4B); *c*-CS (Timstein 4A); *d* – CS (Timstein 4D); *e* – Timstein. **E** Chromosomal control of AMP-2. *a* – CS; *b* – CS (*T. spelta* 4B); *c* – CS (*T. spelta* 4A); *d* – *T. spelta*. **F** Chromosomal control of AMP-2. *a* – Synthetic; *b* – CS (synthetic 4B); *c* – CS (Synthetic 4A); *d* – CS (Synthetic 4D); *e* – CS

Two alien chromosomes can be shown to carry *Amp-1* homoeoloci on their respective group 6 homoeologous chromosomes: the barley *Amp-1* product focusses on the basic side of that of *Amp-B1*, and has already been located on chromosome 6 (=6H) (Hart et al. 1980), while *Amp-Ag^{i}1*, present on the *Ag. intermedium* chromosome shown to carry a number of markers diagnostic for homoeologous group 6 (Figueiras et al. 1987; Forster et al. 1987), codes for an isozyme which focusses on the acidic side of the *Amp-D1* product (Fig. 1 C). A further homoeolocus is present on an as yet unidentified chromosome of *Ag. junceum* (see below).

Chromosomal control of and polymorphism for Amp-2

AMP-2 is strongly active against lysyl and arginyl, and weakly so against methionyl βNAs. As with AMP-1, embryo extracts gave the highest activity; endosperm tissue was satisfactory when extracted in 10 m*M* DTT, although the patterns differed from embryo extracts in a

particular way, as described below. Leaf extracts focussed rather poorly, but a similar pattern to that given by embryo extracts could be recognised.

In the absence of DTT, the AMP-2 pattern of CS consists of three isozymes, focussing between pH 4.15 and 4.25, the central one of which is always more intensely stained than the outer two (Fig. 2A, track a). When the nullisomic-tetrasomics and ditelosomics of CS were assayed, it was shown that the most cathodal isozyme was missing in the absence of chromosome 4A, but was restored in DT4AL (tracks c–e). Examination of the patterns showed that the lack of the short arm of 4D resulted in the disappearance of the more anodal part of the middle band, while the lack of its long arm resulted in the restoration of the euploid pattern (tracks g and h). The pattern of nullisomic 4D-tetrasomic 4B appeared similar to that of CS, as the extra dosage of 4B was responsible for an increase in the 4B-encoded product which forms part of the middle isozyme in CS. The third isozyme behaved rather inconsistently, and its chromosomal control could not be tied to any particular chromosome. The aneuploid analysis thus suggests the presence of a pair of homoeoloci, *Amp-A2* and *Amp-D2,* on 4AL and 4DS, respectively: these arms are known to share homoeology from both cytological and biochemical evidence (reviewed in Worland et al. 1987). These homoeological relationships would predict an *Amp-B2* gene on chromosome arm 4BS, and the pattern of DT4BS appears indistinguishable from euploid, as expected (track b); DT4BL and lines nullisomic for 4B are not available as they are male sterile. However, other lines of evidence demonstrate the presence of an *Amp-B2* gene on 4B (see below).

An examination of the AMP-2 patterns of a range of wheat genotypes shows that the number of isozymes apparently varies from three to six (Fig. 2B). Synthetic shows six distinct AMP-2 isozymes; the four isozymes of higher pI are also present in the *T. dicoccum* parent and the remaining two in the *Ae. squarrosa* parent of this amphiploid (Fig. 2C). Thus, the D genome has contributed two isozymes and the A and B genomes four, and the isozymes appear to act as monomers. The range in band number among hexaploid wheats and the non-disappearance of two of the three CS bands among the aneuploids suggest either the presence of null alleles in CS and in other genotypes showing less than six isozymes, or that some of the bands represent co-focussed isozymes.

The chromosomal control of some of the variant isozymes among wheat genotypes was elucidated by analysis of single chromosomal intervarietal substitutions. Timstein differs from CS in two respects: firstly, by the presence of two novel isozymes focussing on either side of the CS *Amp-A2* product, and secondly, by the loss of the more cathodal part of the co-focussed complex represented by the CS middle band (Fig. 2D, track e). Both of these differences were reproduced in the line CS (Timstein 4B), while both the 4A and the 4D substitutions were identical to CS (tracks b–d). This demonstrates the presence of an *Amp-B2* gene in CS (*Amp-B2a*), shows that part of the CS middle band represents one of its products and locates this gene to the short arm of 4B, since DT4BS has the euploid pattern. Furthermore, it demonstrates that *Amp-B2b,* at least, produces two isozymes (like *Amp-D2*). A third *Amp-B2* allele is present in Hope (*Amp-B2c*), coding for the two isozymes focussing either side of the most anodic CS isozyme (Fig. 2B, track c).

Only one variant was uncovered for *Amp-A2.* In *T. spelta,* the most cathodal product is resolved into a doublet, the CS cathodal isozyme (*Amp-A2a* encoded) is absent and a new, close doublet isozyme appears, which focusses between the *Amp-A2a* product and the CS middle 'isozyme' (Fig. 2B, track f). Part of this pattern can be explained by the presence of *Amp-B2b* (Fig. 2E, track b), but inspection of the pattern of CS (*T. spelta* 4A) shows the presence of the anodal bands of both of the *T. spelta* doublets mentioned above (*Amp-A2b*) (track c).

The D genome isozymes of Synthetic were expressed, as expected, in CS (Synthetic 4D) (Fig. 2F, track d), so that two *Amp-D2* alleles – *Amp-D2a* (CS) and *Amp-D2b* (Synthetic) – could be defined.

Amp-2 homoeoloci are present in a number of alien relatives of wheat. Thus, rye cv Imperial chromosome arm 4RL carries *Amp-R2* (Fig. 3, tracks b–d), the *H. chilense* chromosome $4H^{ch}$ in addition line F *Amp-*H^{ch}*2* (track e), *Ag. intermedium* chromosome *4*Ag^{i} *Amp-*Ag^{i}*2* (tracks f, g), *Ae. sharonensis* chromosome $4S^{l}$ *Amp-*S^{l}*2* (track i), barley chromosome 4 (=4H) *Amp-H2* (tracks j, k), *Ag. elongatum* chromosome 4E *Amp-E2* (track l), *Ag. junceum* chromosome 4J *Amp-J2* (tracks n and o) and *Ha. villosa* chromosome 4V *Amp-V2* (track q). In most of these cases (rye, barley, *Ag. intermedium, Ae. sharonensis*), the alien homoeologue clearly produces two isozymes, just as do the wheat chromosomes; the exceptions appear to only produce one, but it is considered likely that a second product is present but co-focusses with a wheat product and does not, therefore, result in a novel band. These chromosome locations provide further confirmation of the homoeological relationships between these alien chromosomes (and in the case of rye, chromosome arms) and the group 4 wheat chromosomes. Note that a novel AMP-1 isozyme is expressed in the wheat × *Ag. junceum* amphiploid, focussing at a pI slightly greater than the CS *Amp-B1* product (track n).

The presence of two protein products encoded by gene(s) on a single chromosome arm raised the possibility that the *Amp-2* 'loci' comprise not a single gene, but instead two (linked or unlinked) genes. To investigate this, 304 single-seed descent lines derived from the cross

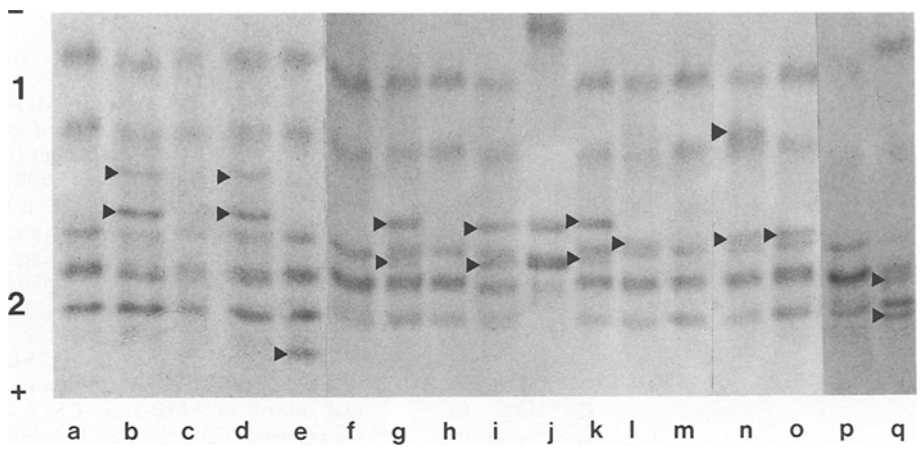

Fig. 3. Alien homoeoloci of *Amp-2*. *Arrowheads* mark isozymes not present in CS. 1, 2 denote the activity of AMP-1 and AMP-2, respectively. *a* – CS; *b* – CS + 4R (Imp); *c* – CS + 4RS; *d* – CS + 4RL; *e* – CS + $4H^{ch}$; *f* – Vilmorin 27; *g* – V27 + $4Ag^{i}$; *h* – CS; *i* – CS + $4S^{l}$; *j* – Betzes; *k* – CS + 4H; *l* – CS + 4E; *m* – CS; *n* – CS × *Ag. junceum* amphiploid; *o* – CS + 4J; *p* – CS; *q* – CS + 4V

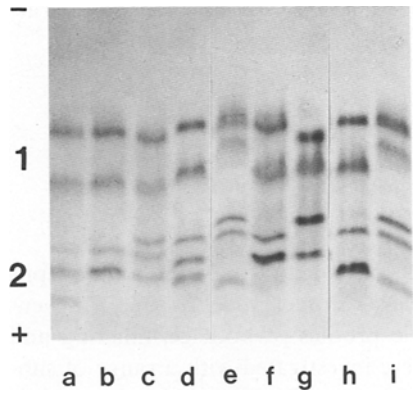

Fig. 4. Effect of DTT on AMP-2 patterns. 1, 2 denote the activity of AMP-1 and AMP-2, respectively. *a* – zero DTT, *b*–*i* – 10 m*M* DTT. *a, b, f*-CS; *c* – Timstein; *d* – Hope; *e* and *i* – Synthetic; *g* – *T. spelta*; *h* – Cheyenne

Sportsman (*Amp-B2a*) × Highbury (*Amp-B2b*) were screened. If the '*Amp-B2* locus' consists of two distinct genes separated by a significant genetic distance, recombinant types showing 4 AMP-2 isozymes should have been found. However, all lines showed either three or five isozymes (156 *Amp-B2a* and 148 *Amp-B2b*) and thus it was concluded that, for marker purposes, a single locus per genome determines AMP-2 activity, and that if two genes are present at this locus, they are tightly linked. However, other evidence described below suggests that only a single gene is present at each of these loci.

In the presence of DTT in the sample extract, the AMP-2 patterns were modified in a particular way. In CS, the most anodal isozyme was not expressed, leaving a pattern of two bands (Fig. 4, tracks a and b). Similarly, the number of bands making up the patterns of other genotypes was reduced: Timstein and Hope showing three bands (reduced from five; tracks c, d), *T. spelta* two bands (from six; track g), Synthetic three bands (from six; track e) and Cheyenne two bands (from five; track h). In all cases, the band(s) absent in these modified patterns represent the higher pI isozyme of the two associated with each locus and, in general, the overall intensity of the patterns is stronger in DTT extracts compared to non-DTT extracts. This behaviour can best be interpreted by assuming that a single gene is present at each locus and that its product is partially oxidised in the absence of DTT.

Chromosomal control of and polymorphism for Amp-3 and Amp-4

AMP-3 showed activity against methionyl, phenylalanyl and tryptophanyl βNAs, and AMP-4 against the latter two of these substrates. The CS AMP-3 pattern consisted of two isozymes, focussing slightly on the acidic side of AMP-2. Analysis of the group 7 aneuploids of CS showed that lines lacking 7A or its short arm showed a less intense cathodal isozyme (Fig. 5 A), locating the gene to chromosome arm 7AS. Among the wheat genotypes tested, only one variant pattern was observed in which the cathodal isozyme was reduced in intensity, and the anodal one was replaced by a pair of significantly more anodal isozymes, as exemplified by Timstein (Fig. 5 B, track e). This pattern was reproduced by CS (Timstein 7A) (track b). A barley homoeolocus could be located on chromosome 1 (=7H) by analysis of the wheat/barley addition lines (see below); with two members of a homoeoallelic series identified, these loci can be assigned the symbols *Amp-A3* (CS has allele *Amp-A3a,* Timstein *Amp-A3b*) and *Amp-H3*.

AMP-4 activity, focussing at a more acidic pI to the *Amp-3* products, is diffuse and was not readily resolvable on these gels. Aneuploid analysis was unsuccessful in assigning the chromosomal control of these isozymes. However, phenylalanyl βNA was a more effective substrate than methionyl βNA for showing the activity of *Amp-H3* (Fig. 3 C). This, along with its weak activity against methionyl βNA, suggests the possibility that the latter gene is instead part of an *Amp-4* homoeoallelic

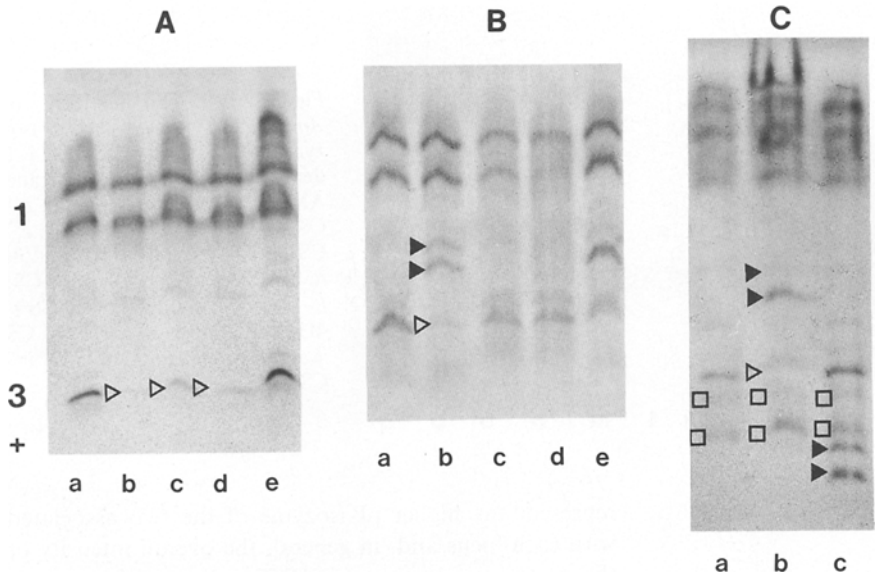

Fig. 5 A–C. AMP-3 and AMP-4 patterns visualised by IEF. *Closed arrowheads* mark bands not present in Chinese Spring (CS), *open arrowheads* the absence of CS bands. 1, 3 denote the activity of AMP-1 and AMP-3, respectively. *Open squares* mark AMP-4 activity. **A** Aneuploid analysis. *a* – CS; *b* – Nullisomic 7A tetrasomic 7B (N7AT7B); *c* – N7AT7D; *d* – Ditelosomic 7AL (DT7AL); *e* – DT7AS. **B** Chromosomal control of AMP-3. *a* – CS; *b* – CS (Timstein 7A); *c* – CS (Timstein 7B); *d* – CS (Timstein 7D); *e* – Timstein. **C** Barley AMP-3, assayed with phenylalanyl βNA. *a* – CS; *b* – Timstein; *c* – CS + 7H

Table 1. Allelic constitution at *Amp-1, -2, -3* loci of wheat genotypes shown in Fig. 2B

Genotype	*Amp-B1*	*Amp-D1*	*Amp-A2*	*Amp-B2*	*Amp-D2*	*Amp-A3*
CS	a	a	a	a	a	a
Bersée	a	a	a	a	c	a
Hope	a	a	a	c	a	a
Timstein	a	a	a	b	a	b
Cheyenne	a	a	a	c	b	a
T. spelta	b	a	b	b	a	a
T. macha	a	a	b	c	c	a
Synthetic	a	b	a	b	b	a

Table 2. Number of alleles detected at the *Amp* loci in wheat and its relatives

	Homoeologous group	Genome			Alien genomes
		A	B	D	
Amp-1	6	1	2	2	H Ag^i
Amp-2	4	2	3	3	H R H^{ch} Ag^i J S^l EV
Amp-3	7	2	–	–	H

series; however, wheat AMP-4 activity is absent in gels stained with the above substrate, and its gene location matches the homoeologous group of *Amp-A3*.

The allelic constitution of the genotypes shown in Fig. 2B, as deduced from analysis of chromosome substitution lines where possible, or otherwise by inference, is given in Table 1. The homoeologous group location, along with the number of alleles found at the various wheat and alien loci are summarised in Table 2.

Discussion

Two previous reports have dealt with wheat aminopeptidases. In the first case (Kruger and Preston 1978), enzyme electrophoretic profiles of both germinating and developing grain were investigated with a range of substrates (leucyl, methionyl, phenylalanyl and tyrosinyl βNAs) using IEF as the means of separation. Differences in relative band intensity were observed, but no band number or pI differences between substrates were described. The reasons for this disparity are unclear, although they may be connected with the extraction procedure followed, where 120 µl of acetate buffer per whole seed was used, a more dilute solution, particularly in the absence of a reducing agent, than was used in the present study for AMP-2 and -3. Thus, the above workers may have only seen the activity of AMP-1 on their gels.

In the second case (Wates and Dalling 1979), a different mode of separation was employed (PAGE – polyacrylamide gel electrophoresis), and the enzyme electrophoretic profile from endosperm and scutellum gave two bands when the gels were incubated with both leucyl and phenylalanyl βNA. Inspection of the published leaf enzyme profiles shows that the patterns obtained with leucyl βNA and phenylalanyl βNA differ from one another in that, in the former case, both bands are equally intense, while in latter, the more anodic band is the more intense. In IEF separations, AMP-3 focusses at a more anodic pI than AMP-1. The likelihood is, therefore, that PAGE is unable to resolve AMP-1 from AMP-3. In addition, these workers point to, but reject on the basis of electrophoretic mobilities, the possibility that wheat possesses an enzyme that acts exclusively against arginyl and

lysyl βNA. It is probable that this enzyme is AMP-2, but that the resolution by PAGE is insufficient to confirm this.

The present study has demonstrated the presence in wheat and some of its relatives, of at least three distinct aminopeptidases, based on substrate specificity, electrophoretic characteristics and genetic control. One of these (AMP-3) may well be the wheat version of Mikola and Mikola's (1986) plant neutral aminopeptidase, characterized by high activity towards phenylalanyl, tyrosinyl, leucyl and methionyl βNAs, but with low or no activity towards arginyl, lysyl, alanyl and glycyl βNAS. In rice (Wu et al. 1988) and maize (Ott and Scandalios 1978), four different enzymes have been established. On the basis of substrate specificity it may be possible to propose the relationships between some of these heterologous enzymes, although biochemical characterization of purified proteins may be necessary before unequivocal conclusions can be arrived at. Thus, maize AMP-1 and rice AMP-1, both of which favour the βNAs of arginine and, to a lesser extent, leucine, may be heterologous with wheat AMP-2; similarly, rice AMP-3 with wheat AMP-1, and maize AMP-4 with wheat AMP-3. Maize AMP-2 is present only in developing endosperm and is assayed with glycyl or alanyl βNA – attempts to find an equivalent in wheat were not successful.

The effect of the reducing agent DTT on the AMP-2 patterns is of interest. This compound is generally added to crude protein extracts to disrupt disulphide bonding, which can produce multiple aggregated forms of particular isozymes, giving a smeared pattern when separated electrophoretically. In the case of AMP-2, the non-reduced extract results in two distinct forms of each isozyme. The regular modification (coalescence of a high and a low pI protein into a single low pI protein) implicates chemical reduction by a single charge unit. It may be of relevance to note that during the purification of barley leucine aminopeptidase, the enzyme was observed to become labile in the absence of sulfhydryl compounds such as DTT (Sopanen and Mikola 1975). In the present case it may, therefore, be that the high pI isozyme represents a partially deactivated form as a result of sulfhydryl bonding. However, there is no consistent pattern of the higher pI isozyme of the pair showing the lesser activity of the two, as measured by staining intensity of the bands on the gels. Alternatively, the two forms represent the in vivo state and are not an artefact of extraction.

The polymorphisms of both *Amp-2* and *Amp-3* are currently being exploited to map these genes on to their respective chromosomes, along with other biochemical, molecular and morphologhical markers (Chao et al. 1989; F. J. Nicoll and M. D. Gale, unpublished results), while *Amp-S*^{1}*2* from *Ae. sharonensis* has been exploited in cytogenetic manipulations of the 4S^{l} cuckoo chromosome (I. P. King, personal communication).

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