

GENE EXPRESSION IN ALLOPLOIDS: GENETIC CONTROL OF LIPOPUROTHIONINS IN WHEAT¹

R. FERNANDEZ DE CALEYA, C. HERNANDEZ-LUCAS, PILAR CARBONERO AND F. GARCIA-OLMEDO

Departamento de Bioquímica, E.T.S. Ingenieros Agrónomos, Madrid-3, Spain

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ABSTRACT

Lipopurothionins are complexes of basic polypeptides and polar lipids found in petroleum ether extracts of wheat endosperm. Location of the structural genes for the protein moiety and of genes probably controlling the lipid moiety has been achieved by analysis of compensated nulli-tetrasomic and ditelosomic lines of *Triticum aestivum* L. cv. Chinese Spring, as well as of other genetic stocks. There are two electrophoretic variants of the apoprotein designated α and β purothionins. Structural genes for α purothionins are located in the long arm of chromosomes 1B and 1D, and for the β variant in the long arm of 1A. These genes have been tentatively designated *Pur-A1*, *Pur-B1*, and *Pur-D1*. The aminoacid composition of purified α and β purothionins from *Triticum aestivum* (genomes AABBDD) and *T. durum* (AABB), and of β purothionin from *T. monococcum* (AA) is also consistent with this conclusion and suggests that the α purothionin encoded by gene *Pur-B1* probably differs from that encoded by gene *Pur-D1* in at least three positions of the aminoacid sequence. A gene (or genes) located in the short arm of chromosome 5D markedly affects the level of lipopurothionin but does not affect apoprotein synthesis. It is concluded that they control the lipid moiety which is required for solubility in petroleum ether.

THE process of allopolyploid (allopolyploid) formation has occurred widely in the evolution of higher plants. Indeed, many plant species that are functional diploids are of allopolyploid origin. Research on the morphological, physiological, cytogenetical and ecological implications of allopolyploidy has been actively pursued (see STEBBINS 1971, for a discussion). Studies at the biochemical level are relevant to our understanding of the evolutionary significance of allopolyploidy in at least three aspects: (a) confirmation of the allopolyploid origin, by identifying genome-specific gene products; (b) assignment of the chromosomal location of genes for different biochemical systems, which can yield valuable clues about secondary modifications undergone by allopolyploids and, especially, about the degree of diploidization of genetic information; and (c) characterization of genome interactions.

Species of the *Aegilops-Triticum* group constitute a well defined allopolyploid complex, of which a great variety of aneuploid and alien substitution stocks is available, and, therefore, are particularly suitable to investigate different problems related to allopolyploidy.

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We report here on the genetic control of a complex biochemical system, the lipopurothionins, in allohexaploid wheat, *Triticum aestivum* L.

Lipopurothionins are protein-lipid complexes, discovered in wheat endosperm by BALLS, HALE and HARRIS (1942), which can be extracted with petroleum ether. Later it was found that the protein moiety consisted of at least two components, designated α and β purothionins (NIMMO, O'SULLIVAN and BERNARDIN 1968; FISHER, REDMAN and ELTON 1968; GARCIA-OLMEDO, SOTELO and GARCIA-FAURE 1968). The lipid associated with these proteins has not been fully identified but there is evidence that it contains phosphate and carbohydrate (BALLS, HALE and HARRIS 1942; REDMAN and FISHER 1968). CARBONERO and GARCIA-OLMEDO (1969) studied the occurrence of α and β purothionins in species of the *Aegilops-Triticum* group and found that diploids had only one of the forms and that only some allopolyploids possessed the two forms. The evidence suggested that each of the three genomes of *T. aestivum* L. has at least one purothionin gene. Also, it was observed by GARCIA-OLMEDO, SOTELO and GARCIA-FAURE (1968) that there were significant differences in lipopurothionin content among the different wheat species. In particular, the yield of crude purothionin in hexaploid wheat (*T. aestivum* L.) was found to be considerably higher than in tetraploid wheat (*T. durum* Desf.).

Based on these preliminary observations, a more detailed study of the genetic control of lipopurothionins was carried out.

MATERIALS AND METHODS

Wheat stocks

Compensated nulli-tetrasomic lines and ditelosomic lines derived from the *Triticum aestivum* (genomes AABBDD) cv. Chinese Spring were obtained from E. R. SEARS (Columbia, Missouri) (SEARS 1954, 1966). The four nulli-tetrasomic combinations involving nullisomy for 2A and 4A were not available. *T. dicoccum* (AABB) TDC-1, *Aegilops squarrosa* (DD) AS-2 and IGC (*T. dicoccum* + 5D5D from *Ae. squarrosa*) were given by T. MELLO-SAMPAYO (Oeiras, Portugal). Tetraploid wheats obtained by extraction of the D genome from the hexaploid cultivars Thatcher, Prelude and Rescue were the gift of P. J. KALTSIKES (Winnipeg, Canada) (KALTSIKES, EVANS and BUSHUK 1968). A synthetic *T. spelta* (AABBDD), obtained from *T. carthlicum* TC-1 (AABB) and *Ae. squarrosa* (DD) AS-1, and the parental lines were provided by E. SANCHEZ-MONGE (Madrid, Spain). The *T. monococcum* (AA) AP line was obtained from M. ALONSO-PEÑA (Cuenca, Spain). *T. aestivum* cv. Aragon 03 and *T. durum* cv. Senatore Capelli and Bidi 17 were originally obtained from Servicio Nacional de Cereales (Madrid, Spain).

Purothionin extraction and purification

For preparative purposes, crude purothionins were obtained from *T. aestivum* cv. Aragon 03, *T. durum* cv. Senatore Capelli and Bidi 17, and *T. monococcum* AP line. Kernels were milled without preconditioning in a Brabender Quadrumat mill and 60–65% yields of milled endosperm (flour) were obtained. The flours (0.8–1.0 Kg) were extracted with 4 volumes of petroleum ether (bp 50–70°) in a glass column (10 × 50 cm). Each extract was concentrated *in vacuo* and crude purothionin was precipitated in hydrochloride form as previously described (GARCIA-OLMEDO, SOTELO and GARCIA-FAURE, 1968). Purothionins α and β were purified by ion exchange chromatography following REDMAN and FISHER (1968).

For quantitative electrophoretic analysis, three different extraction procedures were used: (a) Extraction with petroleum ether (bp 50–70°) as described by CARBONERO and GARCIA-OLMEDO (1969), except that a smaller scale was used (extract from 100 mg ground kernels was trans-

ferred to a 1 × 5 mm Whatman no. 3 paper). (b) Extraction with 4 + 2 volumes of 1M NaCl at 20°, followed by precipitation with 12% trichloroacetic acid at 3°. The precipitate was washed overnight with 8 volumes of ethanol at 3°, centrifuged, and air-dried. (c) Extraction with 4 + 2 volumes of 0.05 N H₂SO₄ at 20°. The extract was brought to pH 4.5 with NaOH and the precipitate formed was discarded after centrifugation. An equal volume of 24% trichloroacetic acid was added to the supernatant at 3°. The precipitate formed was washed overnight with 8 volumes of ethanol at 3°, and air-dried after centrifugation.

Electrophoresis and quantitation

Horizontal starch gel electrophoresis was performed in a 0.015 M aluminium lactate/lactic acid buffer, pH 3.2, containing 3M urea, for 2–3 hr at 10 v/cm and room temperature (system I) or in 0.02 M sodium acetate/acetic acid buffer, pH 5.2, for 2 hr at 10 v/cm and room temperature (system II). System I affords a good separation of purothionins from other components present in the 0.05 N H₂SO₄ extract or the 1 N NaCl extract, but gives little resolution of α and β purothionins. System II allows a good separation of α from β purothionin, but there is overlapping of other components from the above mentioned extracts. The two dimensional combination of both systems permits the analytical separation of α and β purothionins from each other and from all other components. The order of migration is α ahead of β in system I and β ahead of α in system II.

Staining of gels was always carried out with water soluble 0.5% Nigrosine (Fluka catalog no. 72470) in methanol-water-acetic acid (5:5:1) for 14–16 hr and destaining with 70% ethanol after rinsing with tap water.

Quantitation of petroleum-ether-extracted purothionins was carried out by densitometry after electrophoresis in system I. Whatman no. 3 paper strips (1 × 5 mm) containing purothionin extracted from 100 mg of ground kernels were inserted in a 15 × 12 × 0.1 cm gel. All samples to be compared were usually inserted in duplicate in the same gel.

Purothionins extracted with 0.05 N H₂SO₄ or with 1 N NaCl were quantitated by densitometry after two-dimensional electrophoresis. The air-dried precipitates, obtained as described in the previous section, were dissolved in a 0.007 M aluminium lactate/lactic acid buffer containing 8M urea (100 μ l/g of ground kernels). Samples of 4 μ l in a 2 × 2 mm paper strip were inserted for the first electrophoretic run, which was performed in system II. A special plastic template with 2 × 2 × 100 mm channels was designated to cast gels for the first run. These gels, cut to about 60 mm long, were inserted for the second run, which was carried out in 27 × 18 × 0.2 cm gels with system I. Up to 4 samples could be run and compared by this procedure.

Reflectance densitometry was performed with a Chromoscan densitometer (Joyce & Loebel), using a 654 nm filter. Densitometer tracings were directly used for quantitative comparisons without correcting for deviations from linear dose response.

Aminoacid analysis

Aminoacid analysis of purified α and β purothionins from *T. aestivum* cv. Aragon 03 and *T. durum* cv. Senatore Capelli and Bidi 17, as well as of β purothionin from *T. monococcum* AP line, were carried out essentially following MOORE and STEIN (1963). Appropriate aliquots were hydrolyzed for 24, 48 and 72 hr in a Thermoblock at 110 ± 1°. Performic acid oxidation was carried out according to HIRS (1967) and the oxidized samples hydrolyzed for 24 hr. at 110 ± 1°. A Jeol JLC-6AH autoanalyzer was used.

Calculation of the minimum molecular weight and the set of analytical values best adjusted to it was carried out according to DELAAGE (1968), using a Hewlett-Packard electronic calculator (model 9810A) with a function inscriptor (model 9862A). Cysteine values were not used for the adjustment.

RESULTS

Electrophoretic patterns of petroleum-ether-extracted crude purothionins from *Aegilops-Triticum* species are shown in Figure 1. In *T. aestivum* (AABBDD),

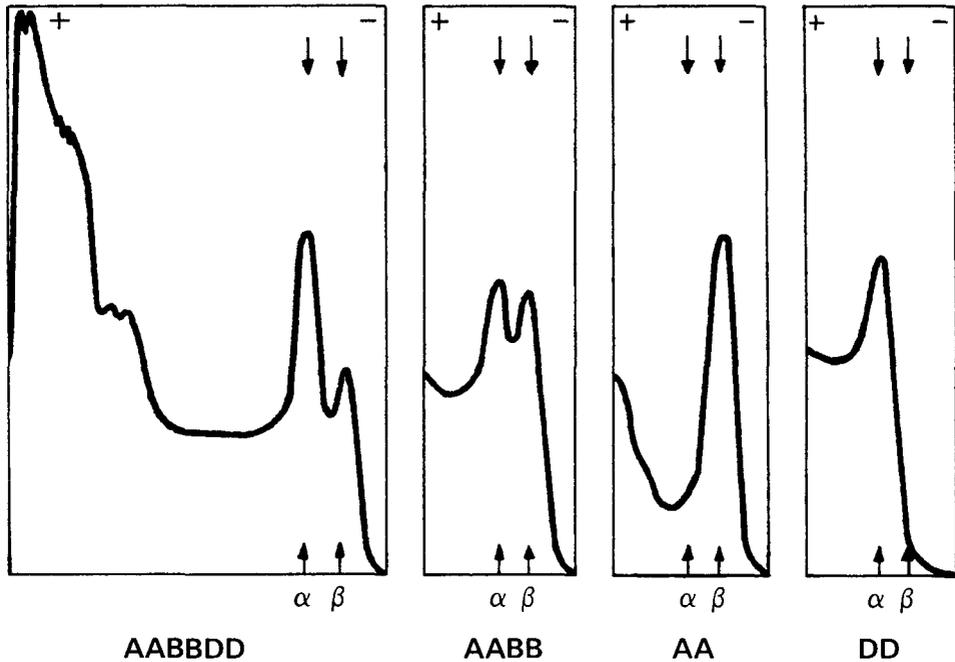


FIGURE 1.—Densitograms of purothionins extracted with petroleum ether and separated by starch gel electrophoresis in 0.02 M sodium acetate buffer, pH 5.2. *Triticum aestivum*, AABBD; *T. durum*, AAB; *T. monococcum*, AA; *Aegilops squarrosa*, DD. In all samples, 10 μ l of a 3 mg/ml crude purothionins solution were applied to a 5 \times 2 mm Whatman no. 3 paper strip and inserted in the gel.

the α : β ratio is about 2:1 while in *T. durum* (AAB) it is about 1:1. Only β purothionin is present in *T. monococcum* (AA) and only α in *Ae. squarrosa* (DD). *Ae. speltoides*, which has been considered as a potential B genome donor, also has only α purothionin [not shown, see CARBONERO and GARCIA-OLMEDO 1969].

Two-dimensional electrophoresis, pH 5.2 \times pH 3.2, of 0.05 N H_2SO_4 or 1 N NaCl extracts of wheat endosperm yields two components that map at the same position as α and β purothionins, respectively. This is illustrated for the 0.05 N H_2SO_4 extract in Figure 2. A comparison of densitograms obtained for the H_2SO_4 extracts of the same species as in Figure 1 are presented in Figure 3. The component migrating as α purothionin was in a 2:1 ratio with that migrating as β purothionin in *T. aestivum* (AABBD), in a 1:1 ratio in *T. durum* (AAB), and was the only one present in *Ae. squarrosa* (DD). Only the component migrating as β purothionin was presented in *T. monococcum* (AA).

As shown in Figure 4, the yield (referred to dry endosperm) of petroleum-ether-extracted purothionins is about 1/10 that of the equivalent components extracted with H_2SO_4 . The yield of the equivalent components from the NaCl extract is somewhat lower than from the H_2SO_4 extract. The salt extract was only

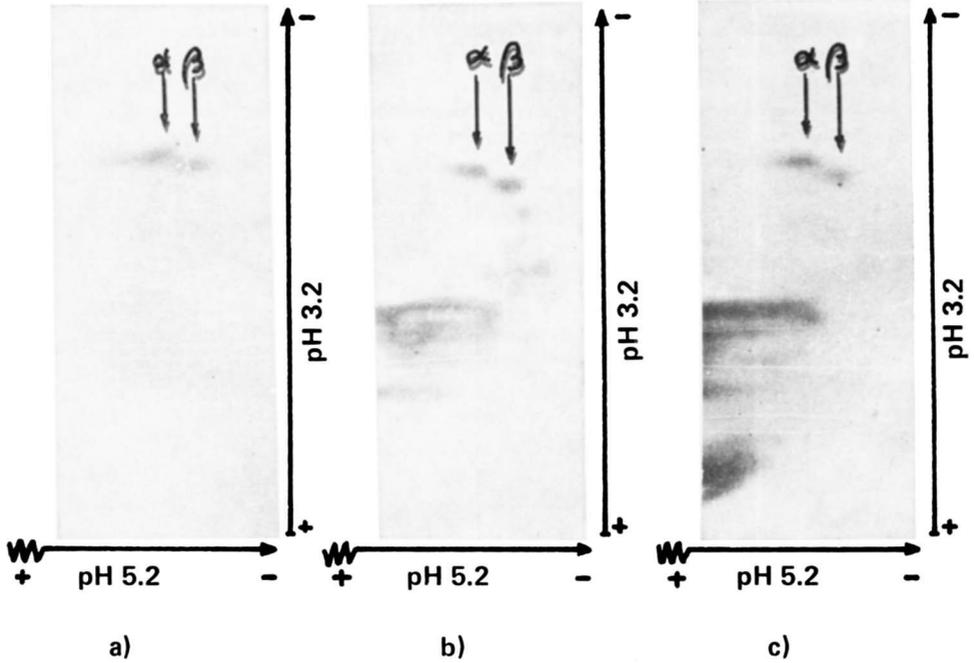


FIGURE 2.—Two dimensional electrophoretic separation of α and β purothionins from *T. aestivum*. (a) Protein extracted with petroleum ether; (b) Protein extracted with 0.05 N SO_4H_2 ; (c) Mixture of (a) and (b). First electrophoresis: 0.02 M sodium acetate buffer, pH 5.2. Second electrophoresis: 0.015 M aluminum lactate buffer, 3M urea, pH 3.2.

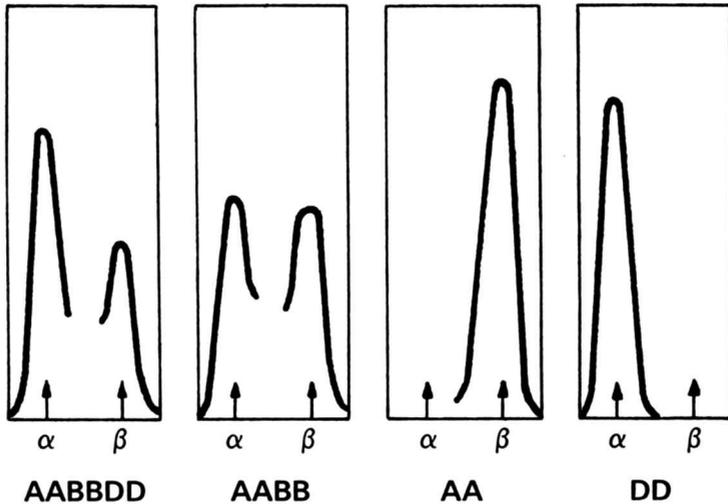


FIGURE 3.—Densitograms of purothionins extracted with 0.05 N SO_4H_2 and separated by two dimensional electrophoresis (0.02 M sodium acetate buffer, pH 5.2 \times 0.015 M aluminum lactate buffer, 3M urea). *Triticum aestivum*, AABBD; *T. durum*, AAB; *T. monococcum*, AA; *Aegilops squarrosa*, DD.

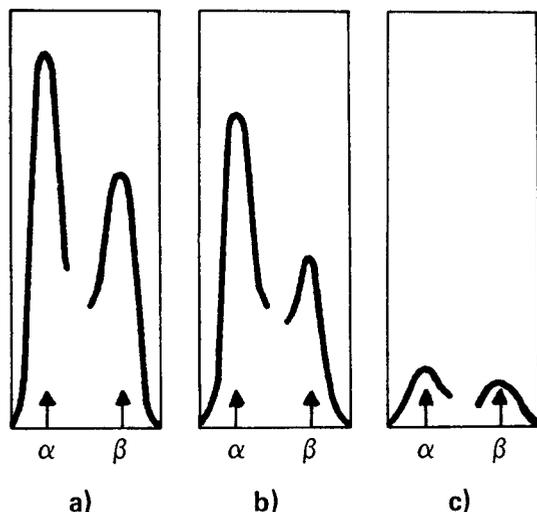


FIGURE 4.—Relative yield of purothionins extracted from *T. aestivum* by different procedures. (a) Extraction with 0.05 N SO_4H_2 ; (b) extraction with SO_4H_2 after petroleum ether; (c) extraction with petroleum ether. Separation of α and β purothionins was carried out by two dimensional electrophoresis (0.02 M sodium acetate buffer, pH 5.2 \times 0.015 M aluminum lactate, 3M urea, pH 3.2 buffer). Densitograms in (a) and (b) correspond to the same amount of endosperm; that in (c) represents a $4 \times$ greater amount. Scanning was carried out in the second electrophoretic direction.

partially soluble in 0.007 lactate buffer, 8 M urea, so this extraction procedure was not used in subsequent quantitative analysis.

An equal weight (100 mg) of ground kernels from each nulli-tetrasomic line was extracted with petroleum ether and purothionins were quantitated by densitometry after electrophoresis at pH 5.2. Observed phenotypes were essentially the same as that of the euploid, except in nulli-tetrasomics of groups 1 and 5.

The electrophoretic patterns shown in Figure 5 are consistent with location of the structural genes for α purothionins in chromosomes 1B and 1D and for β purothionin in chromosome 1A. Not shown are the electrophoretic patterns corresponding to the ditelosomics 1AL, 1BL, and 1DL, which were found to be identical with that of the euploid, indicating that these structural genes are located in the long arms of chromosomes belonging to homoeologous group 1. We tentatively designate *Pur-A1*, *Pur-B1* and *Pur-D1* this set of three homoeoallelic genes.

The marked difference in yield of petroleum-ether-extracted purothionins between *T. aestivum* (AABBDD) and *T. durum* (AABB), which has been already reported (GARCIA-OLMEDO, SOTELO and GARCIA-FAURE 1968), is illustrated in Figure 6-a. Extraction of the D genome from a hexaploid variety results in a marked decrease in yield and in a change in the α : β ratio from 2:1 to 1:1. This was ascertained in the tetraploid wheats obtained from the hexaploid wheats Thatcher, Prelude and Rescue (Figure 6-b). The reverse effect is observed in a synthetic *T. spelta* (AABBDD) obtained from *T. carthlicum* (AABB) and *Ae.*

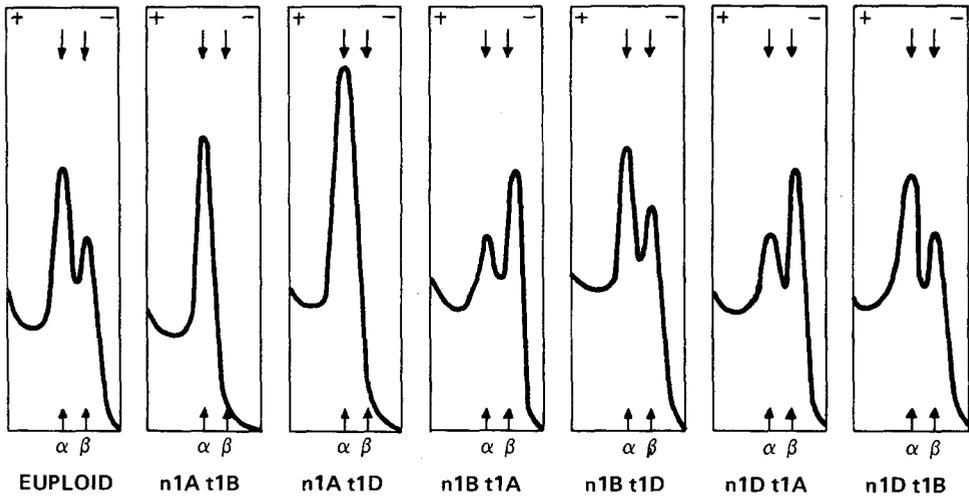


FIGURE 5.—Densitograms of petroleum-ether-extracted purothionins from euploid *T. aestivum* cv. Chinese Spring and nullitetrasomic lines of homoeologous chromosome group 1 (n, nulli; t, tetra). Electrophoresis was carried out in 0.02 M acetate buffer, pH 5.2.

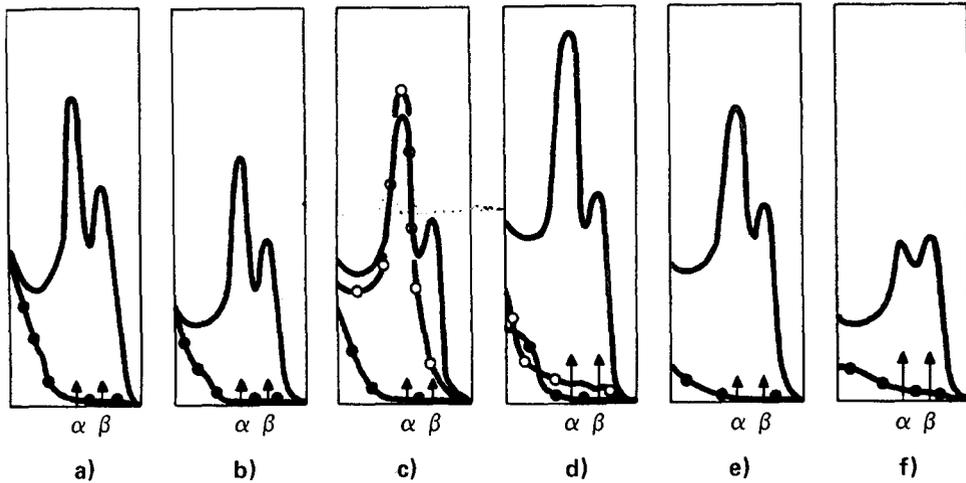


FIGURE 6.—Densitograms of petroleum-ether-extracted purothionins from the following stocks: (a) *T. aestivum* cv. Aragon 03 (—); *T. durum* cv. Senatore Capelli (—●—●—). (b) *T. aestivum* cv. Thatcher (—); tetraploid Thatcher, obtained by extracting D genome from hexaploid Thatcher (—●—●—). (c) *T. carthlicum*, AABB (—●—●—); *Aegilops squarrosa*, DD (—○—○—); synthetic *T. spelta*, AABBDD (—). (d) *T. aestivum* cv. Chinese Spring (—); nulli-5D tetra-5A (—●—●—); nulli-5D tetra-5B (—○—○—). (e) *T. aestivum* cv. Chinese Spring (—); ditelo 5D long arm (—●—●—). (f) *T. dicoccum*, AABB (—●—●—). IGC, disomic addition line of chromosome 5D from *Aegilops squarrosa* to *T. dicoccum* (—). Electrophoresis was carried out in 0.02 M acetate buffer pH 5.2. Densitograms within each panel correspond to the same weight of ground kernels and identical staining conditions.

squarrosa (DD) (Figure 6-c). The electrophoretic profiles in Figures 6-d and 6-e indicate that a gene (or genes) located in the short arm of chromosome 5D are responsible for the effect associated with the D genome. The disomic addition of chromosome 5D from *Ae. squarrosa* to a tetraploid wheat does increase the yield of petroleum-ether-extracted purothionins but does not alter the $\alpha:\beta$ ratio (Figure 6-f). The yields from group 5 nulli-tetrasomics not shown in Figure 6 are approximately as follows: nulli-5A tetra-5B \approx nulli-5A tetra-5D \approx nulli-5B tetra-5D \approx euploid $>$ nulli-5B tetra-5A \approx nulli-5D tetra-5B $>$ nulli-5D tetra-5A.

Figure 7 shows that the ratios of components from H_2SO_4 extracts migrating as α and β purothionins in nulli-tetrasomics of group 1 match the $\alpha:\beta$ ratios of petroleum ether purothionins shown in Figure 5.

Results presented in Figure 8 indicate that chromosome 5D has no effect on yields or $\alpha:\beta$ ratios of components from H_2SO_4 extracts migrating as α and β purothionins.

The aminoacid composition of purothionins purified from the petroleum ether extracts of *T. aestivum* (AABBDD), *T. durum* (AABB) and *T. monococcum* (AA) is presented in Table 1. The number of aminoacid residues per mole, corresponding to the calculated minimum molecular weight, was 43 for α purothionin from *T. durum* and for β purothionin from the three wheat species. In all cases the analytical values of phenylalanine best adjusted to the minimum molecular weight were 1.0. The experimental values given for half-cystine are the half-cystine : phenylalanine molar ratios obtained from the unoxidized samples, which were higher than those obtained after performic acid oxidation. These

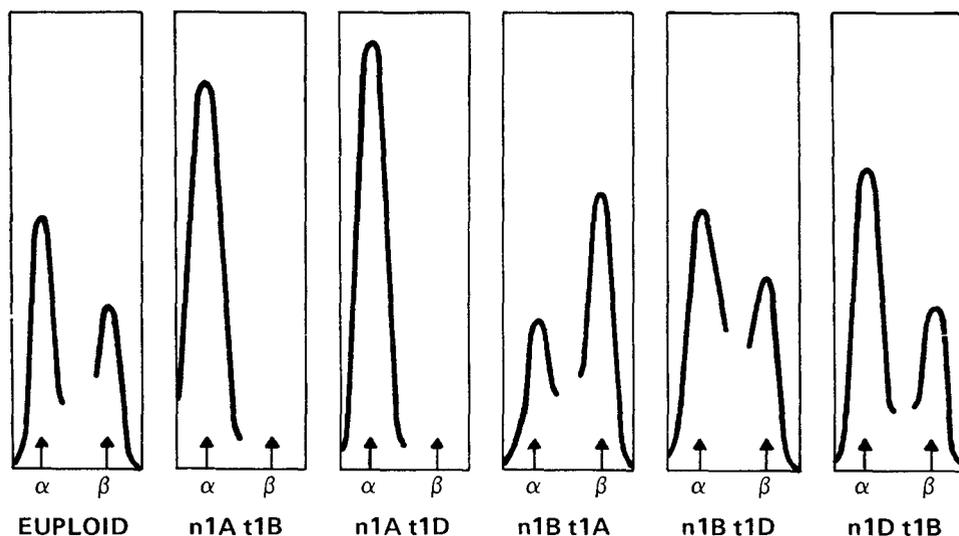


FIGURE 7.—Densitograms of 0.05 N SO_4H_2 extracted purothionins from euploid *T. aestivum* cv. Chinese Spring and nullitetrasomic lines of homoeologous chromosome group 1 (n, nulli; t, tetra). Separation was carried out by two dimensional electrophoresis (0.02 M acetate buffer, pH 5.2 \times 0.015 M aluminium lactate buffer, 3M urea, pH 3.2).

TABLE 1
Estimated number of aminoacid residues for minimum molecular weight of α and β purothionins from different wheat species

Aminoacid	α Purothionins						β Purothionins					
	<i>T. durum</i> (α_B)		<i>T. aestivum</i> ($\alpha_B + \alpha_D$)		<i>T. aestivum</i> (α_D)		<i>T. monoccoccum</i>		<i>T. durum</i>		<i>T. aestivum</i>	
	Found	Nearest integer	Found	Nearest half mole	Extra-polated	Nearest integer	Found	Nearest integer	Found	Nearest integer	Found	Nearest integer
Lysine	3.7	4	3.9	4		4	5.1	5	4.8	5	4.7	5
Arginine	3.8	4	3.9	4		4	3.0	3	3.3	3	3.3	3
Aspartic	1.7	2	2.3	2.5	2.9	3	4.1	4	3.0	3	3.6	4
Threonine	1.1	1	2.2	2	3.3	3	1.8	2	1.8	2	2.0	2
Serine	5.8	6	5.3	5.5	4.8	5	4.2	4	4.4	4	4.4	4
Glutamic	1.4	1	1.3	1		1	1.8	2	1.6	2	1.6	2
Proline	1.7	2	1.9	2		2	2.1	2	2.0	2	1.7	2
Glycine	4.3	4	4.2	4		4	3.1	3	3.6	4	3.3	3
Alanine	2.7	3	2.3	2.5	1.9	2	3.3	3	3.1	3	2.8	3
Cystine/2	7.3	8	6.9	8		8	7.0	8	7.5	8	6.7	8
Valine	1.0	1	1.1	1		1	1.2	1	1.1	1	1.1	1
Isoleucine	0.9	1	0.5	0.5	0.1	0	0.4	0	0.4	0	0.3	0
Leucine	3.8	4	3.8	4		4	4.4	4	3.9	4	4.4	4
Tyrosine	0.6	1	0.6	1*		1	0.7	1	0.9	1	0.6	1
Phenylalanine	1.0	1	1.0	1		1	1.0	1	1.0	1	1.0	1
No. of residues		43		43		43		43		43		43
Molecular weight		4,575.6				4,621.5		4,663.5		4,605.7		4,663.5

* Taken as 1 because there was no difference between α_B and $\alpha_B + \alpha_D$.

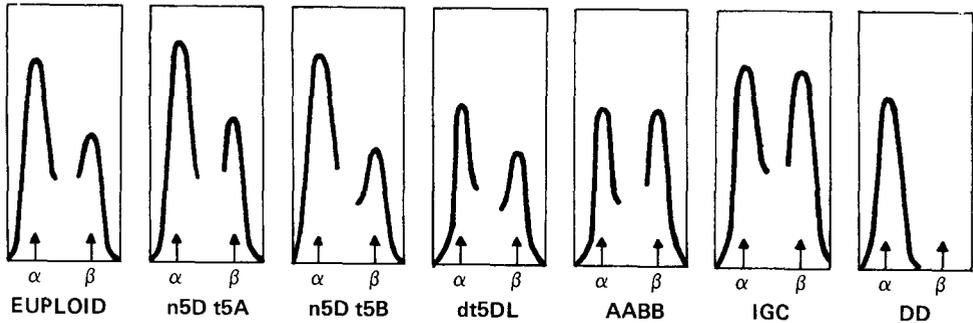


FIGURE 8.—Densitograms of 0.05 N SO_4H_2 extracted purothionins from the following stocks: Euploid *T. aestivum* cv. Chinese Spring; n5D t5A; n5D t5B; dt5DL, chromosome 5D long arm ditelosomic; *T. dicoccum*, AABB; IGC, disomic addition line of chromosome 5D from *Aegilops squarrosa* to *T. dicoccum*; *Ae. squarrosa*, DD. Separation was by two dimensional electrophoresis (0.02 M acetate buffer, pH 5.2 \times 0.015 M aluminium lactate buffer, 3M urea, pH 3.2). All densitograms in figure correspond to the same weight of ground kernels and identical staining conditions.

values are probably an underestimation and since no free sulphhydryl groups are detected in these proteins, 8 was taken as the nearest even number of moles for half-cystine.

The composition of β purothionin from *T. aestivum* was identical with that from *T. monococcum*. However, in β -purothionin from each of the *T. durum* cultivars analyzed there was one less aspartic and one more glycine residue per molecule than in that from *T. aestivum*.

The analytical values given in Table 1 for α purothionin from *T. aestivum* are the number of moles of each aminoacid per mole of phenylalanine. These values coincide with those obtained for α purothionin from *T. durum*, except in the case of aspartic, threonine, serine, alanine and isoleucine. The above genetic evidence and the aminoacid analysis suggest that α purothionin from *T. aestivum* is really a 1:1 mixture of two genetic variants, one from the B genome (α_B) and the other from the D genome (α_D). If the analytical values are approximated to the nearest half mole, instead of the nearest integer, 43 moles of aminoacids per mole of phenylalanine are obtained. Assuming that α_B is identical in *T. durum* and *T. aestivum*, the aminoacid composition of α_D can be deduced by extrapolation. A value of 43 residues per mole of α_D is again obtained, with differences with respect to α_B in at least three positions in the sequence (+1 aspartic, +2 threonines; -1 serine, -1 alanine, -1 isoleucine see Table 1). A comparison of the aminoacid composition of α and β purothionins from *T. durum* yields a minimum of four differences in their sequences (+1 lysine, +1 aspartic, +1 threonine, +1 glutamic; -1 arginine, -2 serines, -1 isoleucine, see Table 1).

DISCUSSION

The above results confirm the previous finding of CARBONERO and GARCIA-OLMEDO (1969) that there is at least one gene for purothionins in each of the

genomes of allohexaploid wheat, and permit the assignment of the structural gene for β purothionin to the long arm of chromosome 1A and the structural genes for α purothionins to the homoeologous arms of 1B and 1D.

The aminoacid composition of α and β purothionins from the three wheat species is also compatible with the genetic evidence and, in our opinion, fully explains the discrepancies in molecular weight calculations from aminoacid composition (REDMAN and FISHER 1968, 1969) and from physical measurements (NIMMO, KASARDA and LEW 1974). Unfortunately, most of the chemical and physical characterization of purothionins carried out previously have dealt with α purothionin from *T. aestivum*, which is really a mixture of two genetic variants. The minimum molecular weight calculated from the aminoacid analysis of this mixture was higher than those derived from physical measurements because the proportion of isoleucine is 1/43 in α_B and 0 in α_D and, thus, in the 1:1 mixture this proportion is 1/86, leading to an overestimation of the minimum molecular weight. NIMMO, KASARDA and LEW (1974) reported molecular weights of 5100–5300 by sedimentation equilibrium, 6100 by osmotic pressure measurements, and about 7500 by gel permeation chromatography. When their data for the aminoacid analysis of α purothionin from *T. aestivum* ($\alpha_B + \alpha_D$) are expressed per mol of phenylalanine, there is complete agreement with ours, except for arginine (5 instead of 4) and lysine (5 instead of 4). The minimum molecular weights calculated by the method of DELAAGE (1968) using our data are in the vicinity of 5000, close to the estimation obtained by sedimentation equilibrium.

NIMMO, O'SULLIVAN and BERNARDIN (1968) demonstrated that after exposure of globulin preparations from wheat endosperm to low pH, a low molecular weight fraction was produced that, when purified, had the same electrophoretic pattern, molecular weight, UV spectrum and aminoacid composition as the light-petroleum extracted purothionin fraction. REDMAN and FISHER (1968) further fractionated the globulin fraction into two components, α and β , whose aminoacid compositions, tryptic peptide maps and C-terminal aminoacids matched those of α and β purothionins. The present genetic evidence (Figures 1–3, 5, 7) further supports the notion that the purothionin-like components obtained by salt extraction and exposure to acid pH, or by direct sulphuric acid extraction, are actually purothionins.

The higher yield of petroleum-ether-extracted purothionins in hexaploid versus tetraploid wheats is dependent on a gene (or genes) located in the short arm of chromosome 5D (Figure 6). However, this chromosome arm has no effect on yield of acid-extracted purothionins (Figure 8). This strongly suggests that what is controlled by the short arm of chromosome 5D is the lipid moiety of lipopurothionins, which is required for solubility in petroleum ether.

While the three apoprotein structural genes seem to be about equally productive, and fully compensate for each other in nulli-tetrasomics, this is not the case for genes located in group 5 chromosomes. These chromosomes could be ranked as to their effectiveness in affecting the yield of lipopurothionin as follows: 5D > 5B > 5A. The present data do not reveal whether or not chromosome 5A contributes at all to lipopurothionin production in hexaploid wheat.

In a preliminary review of part of the data on petroleum-ether-extracted purothionins (GARCIA-OLMEDO *et al.* 1974), the genetic regulation of lipopurothionins was tentatively proposed as an example of intergenomic complementation in the completion of a holoprotein. Although it is known that purothionins inhibit papain (BALLS, HALE and HARRIS 1942b) and have antimicrobial and uterus-contracting properties (STUART and HARRIS 1942; COULSON, HARRIS and AXELROD 1942; FERNANDEZ DE CALEYA *et al.* 1972; HERNANDEZ-LUCAS, FERNANDEZ DE CALEYA and CARBONERO 1974), there are no clues about the physiological functions of either purothionins or lipopurothionins. The present evidence that, even in *T. aestivum*, petroleum-ether-extracted purothionin represents a small fraction (10–15%) of total purothionin and that the group 5 chromosomes do not affect the level of purothionin production, raise the possibility that lipopurothionins are extraction artifacts and do not exist as such *in vivo*.

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Corresponding Editor: J. R. LAUGHANAN