Table III. Free Amino Acid Content of Pecan Nutmeats[a]

amino acid	μmol/g dry wt (SEM)
aspartate	1.7 (0.37)
threonine	0.1 (0.04)
serine	0.4 (0.09)
asparagine	0.2 (0.09)
glutamate	1.6 (0.37)
glutamine	0.4 (0.06)
proline	0.5 (0.04)
glycine	0.2 (<0.01)
alanine	0.5 (0.07)
valine	0.4 (0.25)
methionine	0.1 (0.02)
isoleucine	0.1 (0.02)
leucine	0.1 (0.01)
tyrosine	0.1 (0.01)
phenylalanine	0.1 (0.01)
γ-aminobutyrate	0.1 (0.02)
lysine	0.2 (0.01)
histidine	0.1 (0.01)
arginine	0.4 (0.10)
citrulline	1.4 (0.51)
ornithine	0.1 (0.04)
3-methylhistidine	<0.1
cysteine	Tr
cystathionine	<0.1
β-alanine	Tr
ethanolamine	Tr
ammonia	3.0 (0.55)

[a] Mean content of check (0 N) samples only is presented with standard error of the mean. Tr = trace.

of lysine in the nutmeat, a response similar to those seen in other crops (Elmore et al., 1979). Nitroform, on the other hand, did not affect the lysine concentration. This is probably a consequence of the slow release character of nitroform which provides continued availability of N during the growing season, especially during the nut-filling period. These effects are very important and should be studied further.

This presentation of the free amino acid composition is the first available in the literature for pecans and shows that there are more different kinds of amino acids or amino-containing compounds in pecan than in many other seeds that are used for food. The urea cycle components were quite prominent.

Pecans were shown to be a reasonable source of protein, with acceptable levels of the sulfur amino acids; however, they are limiting in lysine and thus of limited use for monogastric animal nutrition.

ACKNOWLEDGMENT

We thank M. K. Heaton and S. D. Senter for reviewing a prior draft of this manuscript and acknowledge the excellent technical assistance of Louis Clarke and Wesley Long.

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Received for review January 22, 1980. Accepted May 27, 1980.

Purification and Characterization of a Corn (*Zea mays*) Protein Similar to Purothionins

Berne L. Jones* and D. Blake Cooper

Extraction of petroleum ether defatted ground corn (*Zea mays*) with dilute sulfuric acid yielded six protein fractions, each of which contained large numbers of basic amino acid and cysteine residues. All of the proteins appeared to be similar in size and amino acid composition to the purothionins found in wheat flour. One protein was almost identical in amino acid composition to the thionin recently isolated from rye flour. This protein, corn protein I, was hydrolyzed with chymotrypsin and yielded four peptides, none of which resembled the two peptides released from purothionins under identical conditions. None of the six proteins were toxic when injected into tobacco hornworm (*Manduca sexta*) larvae. Even though the corn protein I is very similar to purothionins and rye thionin in amino acid composition and in several chemical and physical properties, it is probably not homologous in primary structure.

Purothionins are a group of small proteins (or large polypeptides) 45 amino acids in length that were isolated from wheat flour (Balls and Hale, 1940). They are very basic (20% Arg + Lys) and contain large amounts of Cys (20%) but no His, Met, or Trp (Nimmo et al., 1974; Redman and Fisher, 1968). The amino acid sequences of the three purothionin species present in bread wheat (*Triticum aestivum*) have been determined (Mak and Jones, 1976a; Jones and Mak, 1977; Ohtani et al., 1975). Wheat purothionins are homologous in structure with a group of toxic mistletoe (*Viscum album*) proteins (Mak and Jones, 1976a; Samelsson et al., 1968; Samuelsson, 1974). Homologous proteins have also been found in various species of the Aegilops and Triticum groups (Carbonero and Garcia-Olmedo, 1969) and have been purified from barley (Redman and Fisher, 1969) and from rye (Hernandez-Lucas et al., 1978). As a group, these

U. S. Grain Marketing Research Laboratory, Agricultural Research, Science and Education Administration, U. S. Department of Agriculture, Manhattan, Kansas 66502.

homologous proteins are referred to as thionins; however, the term purothionins applies only to the three members of this group that occur in wheat. The fact that homologous proteins have been genetically conserved in such diverse species indicates that they are important in plants, althouth their exact functions are unknown. As part of an investigation into the distribution of purothionin homologues in grains, we looked for such proteins in corn and report the results in this article.

MATERIALS AND METHODS

Biological Materials. Whole yellow corn (*Zea mays* L.) was milled on a Weber hammer mill and sifted through a 0.0071-in. mesh sieve.

Extraction and Purification Procedures. Lipids were extracted from ground corn with petroleum ether by the method of Redman and Fisher (1969) as modified by Mak and Jones (1976b). After treatment of the extracted lipid with lactic acid solution, the aqueous fraction was adjusted to pH 5.2 with ammonium hydroxide, loaded onto a carboxymethylcellulose (CM-cellulose) column, and eluted with a 0.3–1.0 M, pH 5.2, ammonium acetate (NH_4OAc) buffer gradient (Mak and Jones, 1976b).

Corn proteins were extracted with sulfuric acid (0.05 M) by a modification of the method of Fernandez de Caleya et al. (1976) for wheat purothionins. Ground corn was defatted with petroleum ether (PE) in a 120 × 12 cm glass column until the eluate was colorless. The PE-extracted corn was removed from the column and dried, and a 2-kg aliquot was put into 4 L of 0.05 M H_2SO_4. The mixture was thoroughly stirred and then incubated for 1 h at room temperature with occasional mixing. The mixture was centrifuged at 850*g* for 6 min, and the supernatant was filtered through a clean towel. The 850*g* pellet was resuspended in 1600 mL of 0.05 M H_2SO_4, stirred, incubated for 1 h, and centrifuged. The supernatant from this second centrifugation was filtered, and the two 850*g* supernatants were pooled. Trichloroacetic acid (Cl_3AcOH) was dissolved in the supernatant (12% w/v), and the resulting suspension was kept at 4 °C overnight. Precipitated protein was recovered by centrifuging the Cl_3AcOH suspension at 850*g* for 10 min. The supernatant was discarded. The precipitated protein was suspended in 200 mL of H_2O, stirred 30 min, and centrifuged at 27000*g* for 10 min. The 27000*g* supernatant was retained while the precipitate was reextracted with an additional 150 mL of H_2O. After centrifugation, the two 27000 *g* supernatants were combined, adjusted to pH 5.2 with NH_4OH, and loaded onto a column (2.6 × 15 cm) of CM-cellulose equilibrated with 0.3 M NH_4OAc, pH 5.2. The column was washed with 0.3 M buffer until all nonbound material absorbing at 280 nm was eluted, and then the proteins of interest were eluted with 1.0 M NH_4OAc buffer, pH 5.2. The 1.0 M eluate was diluted to 0.3 M with H_2O and loaded onto a small (1.2 × 12 cm) CM-cellulose column equilibrated with 0.3 M buffer. The proteins were subsequently eluted with a 900-mL linear gradient, from 0.3 to 1.0 M, of NH_4OAc (pH 5.2) by the method of Mak and Jones (1976b).

Amino Acid Analysis. Amino acids were determined in all protein fractions by the method of Moore and Stein (1963). Aliquots (0.1–1 mg) were hydrolyzed with 6 N HCl for 24 h at 110 °C under vacuum. Phenol was added to the HCl to combat Tyr decomposition in some of the hydrolyses. The hydrolysate was analyzed on a Beckman Model 121 analyzer modified for analysis of small samples yielding ninhydrin color densities of up to 0.5 OD full-scale.

Chymotryptic Peptide Formation. Corn protein fraction I (36 mg) was dissolved in 6 mL of H_2O and adjusted to pH 7.7 with 10 mM NaOH in a Brinkmann pH stat. After addition of 0.9 mg of α-chymotrypsin (Worthington Biochemical, CDIZKD, 67 IU/mg) dissolved in 1.5 mL of 0.5% ammonium bicarbonate buffer, pH 7.7, the reaction was monitored by following the amount of 10 mM NaOH required to maintain pH 7.7. After 20 min 6.2 μequiv of NaOH solution had been added and the reaction was terminated by adjusting the pH to 3.7 with acetic acid. The sample was freeze-dried, reduced, pyridylethylated, loaded onto a 1.2 × 10 cm CM-cellulose column equilibrated with 0.05 M ammonium acetate, pH 5.2, and eluted with a linear 0.05–0.65 M gradient of NH_4OAc buffer, pH 5.2.

Pyridylethylation of Peptides. Disulfide bonds were reduced, and the resulting -SH bonds were pyridylethylated with 4-vinylpyridine by the method of Mak and Jones (1976b).

$NaDodSO_4$ Spot Tests for Thionins. An aliquot (100 μL) of solutions to be tested for thionins was placed in the well of a black spot plate and adjusted to a pH below neutrality (pH 5.2 is good). Sodium dodecyl sulfate ($NaDodSO_4$, 10 μL of a 0.3% w/v solution) was added with stirring. A granular white precipitate formed if thionins were present at 0.5 $\mu g/\mu L$ or above. The precipitate dissolved if a large excess of $NaDodSO_4$ was added and did not form if the pH was much above neutrality. Formation of a precipitate did not necessarily ensure that thionins were present since a few other proteins also are insoluble under these conditions; but all thionins tested to date (α_1-, α_2-, and β-purothionins, several hordothionins, and rye thionin) have given strong precipitates with $NaDodSO_4$.

Toxicity Tests. Each corn protein fraction was bioassayed for toxicity to the tobacco hornworm (*Manduca sexta*) by the procedure of Kramer et al. (1979). Larvae were injected with 100 μg of protein. The NH_4OAc used to elute the proteins was removed by freeze-drying each fraction twice.

Sephadex G-50 Column Chromatography. Sephadex G-50 medium gel was equilibrated with 0.1 M acetic acid and packed into a 1.2 × 87 cm column. Standard protein samples and corn protein samples were dissolved in 0.1 M acetic acid (~2 mg of protein in 0.5 mL) and applied to the column which was developed with 0.1 M acetic acid. The column effluent was monitored at 254 and 280 nm. *K*av values were calculated by the method recommended by Pharmacia Fine Chemicals (1979).

RESULTS AND DISCUSSION

Extraction and Separation. Finely ground corn was extracted with petroleum ether (PE) by a method that yields about 300 mg of purothionin from 4 kg of wheat flour (Mak and Jones, 1976b). With PE no corn protein was extracted that resembled any of the wheat purothionins. That was not unexpected since the purothionin–lipid complex extracted from wheat with PE is probably an artifact (Hernandez-Lucas et al., 1978); possibly corn did not contain the lipid required to complex with any purothionin homologue to render it soluble in PE. Rye flour (Hernandez-Lucas et al., 1978) contained a purothionin homologue, rye thionin, which is insoluble in PE until a wheat lipid fraction is added. After a presumed rye thionin–wheat lipid complex forms, the rye protein can be extracted with PE and purified.

Fernandez de Caleya et al. (1976) extracted purothionin from bread wheat flour with 0.05 M H_2SO_4 and we purified purothionins from durum wheat by a modification of their method (unpublished experiments). We therefore extracted ground corn with H_2SO_4, precipitated proteins with 12% Cl_3AcOH and separated the Cl_3AcOH-precipitated proteins on a CM-cellulose column. The results are shown

Table I. Amino Acid Compositions of Corn Acid-Soluble Proteins and of Wheat β-Purothionin and Rye Thionin

amino acid	corn proteins, mol %						corn protein I[a]	β-purothionin[a,b]	rye thionin[a]	
	I	II	III	IV	V	VI			c	d
Lys	7.1	8.4	8.1	9.1	12.9	4.6	3.4	6	4.5	4.6
His	5.9	5.9	5.7	5.6	5.0	6.5	2.8	0		2.8
Arg	10.5	14.8	19.2	17.5	16.9	24.9	5.0	4	4.3	4.3
Asx	6.9	8.1	7.5	6.7	4.8	2.1	3.1	4	2.8	2.7
Thr	2.1	1.5	1.0	1.1	1.8	2.5	1.0	2	2.2	2.1
Ser	7.8	6.2	5.8	5.4	5.7	3.8	3.7	4	4.2	3.7
Glx	11.5	17.0	22.2	19.1	16.4	22.3	5.5	1	2.8	5.2
Pro	3.4	3.1	2.0	3.2	5.0	4.2	1.6	2	1.7	2.1
Gly	8.6	8.3	6.7	6.5	4.5	9.7	4.1	3	4.2	4.1
Ala	4.6	3.9	3.7	4.2	8.3	1.4	2.2	3	2.3	1.9
Val	4.2	3.5	3.3	3.9	3.7	2.1	2.0	1	0.4	1.1
Met	2.5	2.6	2.7	2.7	2.2	2.6	1.2	0		1.0
Ile	2.1	1.5	0.9	0.9	1.1	0.8	1.0	0	1.1	1.3
Leu	4.6	3.8	2.2	2.2	3.1	3.9	2.2	5	2.9	2.0
Tyr	3.6	2.2	0.5	2.0	Tr	0.0	1.7	1	0.7	0.9
Phe	2.1	1.3	0.5	0.3	0.6	0.5	1.0	1	1.4	1.0
Cys[e]	12.6	10.0	8.6	9.7	7.9	8.2	6.0	8		5.5

[a] Composition based on residues of amino acid per mole of protein. [b] From Mak and Jones (1976a). [c] From Hernandez-Lucas et al. (1978). [d] Data from this laboratory. [e] Cys was probably partially destroyed during hydrolysis; these are minimal values.

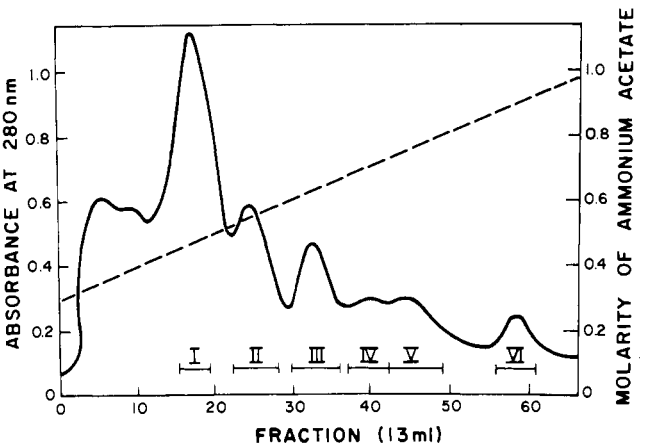

Figure 1. Elution of basic corn proteins soluble in sulfuric acid (0.05 M) from a carboxymethylcellulose column with a linear gradient of ammonium acetate. Numbered bars indicate fractions pooled.

in Figure 1. Six protein fractions were eluted from the CM-cellulose column between 0.4 and 0.9 M NH_4OAc. The peak tube from each fraction was tested to determine the solubility of each protein in the presence of $NaDodSO_4$. Thionins from wheats, rye, and barley, at concentrations over 0.5 μg/μL, formed insoluble $NaDodSO_4$–thionin precipitates under the test conditions (unpublished observations). Two corn protein fractions, I and VI, gave precipitates with $NaDodSO_4$.

Contents of tubes from each absorbing peak were combined as shown in Figure 1 and were freeze-dried twice to remove NH_4OAc. Yields from 2 kg of ground corn were as follows: I, 65 mg; II, 48 mg; III, 35 mg; IV, 19 mg; V, 25 mg; VI, 14 mg. Material eluting prior to fraction and I was tested for toxicity but not investigated further, as it did not precipitate in the presence of $NaDodSO_4$ and overlap of the fractions was too great for meaningful amino acid analysis.

Data in Figure 1 are from the combined supernatants of two consecutive water washes of the 12% Cl_3AcOH precipitate. When the supernatants from the first and second water washes of a single Cl_3AcOH precipitate were eluted separately from CM-cellulose columns, the relative amounts of the six proteins differed greatly. The initial supernatant contained almost no material eluting prior to fraction I and no fraction VI while the second supernatant contained 15 mg of fraction VI but no fraction II protein. Both initial and second washes contained large amounts of fraction I protein.

Amino Acid Compositions. Amino acid compositions of thionins from wheats, barley, and rye differ markedly from most other endosperm proteins, being very high in Cys, Lys, and Arg but low in Glx and Pro. The amino acid compositions of the six protein fractions from corn are shown in Table I along with those of wheat β-purothionin and of rye thionin. Corn proteins I–IV all had similar amino acid compositions except for Arg and Glx, which varied widely. Fractions V and VI, however, differed from the other fractions and from each other in the number of Lys, Asx, Pro, Gly, and Ala residues, as well as in Arg and Glx. When the amino acid data for corn protein I were converted from mole percent into amino acid residues per mole, based on the presence of 1.0 residue each of Phe, Ile, and Thr per mol of protein (Table I), the protein had a minimum size of about 47 amino acid residues, or around M_r 5000. This is very similar to the size of the wheat purothionins (45 residues each; Mak and Jones, 1976a; Ohtani et al., 1975) and of rye thionin (about 43 residues; Hernandez-Lucas et al., 1978). The amino acid composition of fraction I was similar to that of β-purothionin (Table I), but the corn protein contained His and Met, which are never present in purothionins. The compositions of corn protein I and of the rye thionin isolated in our laboratory were very similar. The rye thionin isolated by Hernandez-Lucas et al. (1978) had a composition similar to that of corn protein I but was not reported to contain any His or Met.

Sephadex G-50 Gel Filtration. The physcial sizes of the corn proteins were examined by comparing the positions at which they eluted from a Sephadex G-50 column developed with 0.1 M acetic acid (Figure 2). The molecular weigths of the proteins were not necessarily directly related to the relative molecular sizes measured in this experiment, because the column was not run under completely denaturing conditions and the elution volumes may have been affected by the secondary and/or tertiary structures of the proteins, as well as by their molecular weights. When native (not reduced and pyridylethylated) corn protein fractions were analyzed, they eluted in three

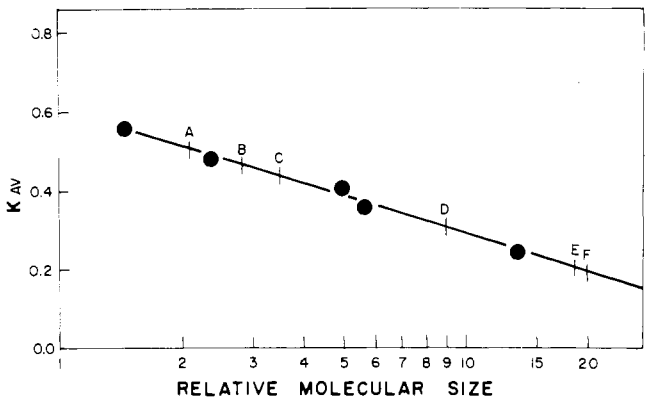

Figure 2. Determination of relative molecular sizes of native and pyridylethylated corn proteins and β-purothionin. Standard and corn proteins were eluted from Sephadex G-50 in 0.1 M acetic acid. Because completely denaturing solvents were not used, the molecular sizes are relative, not absolute. Standard proteins are plotted as K_{av} vs. $M_r/1000$. Protein standards were, from lowest to highest molecular weight bacitracin, insulin A chain, β-purothionin, insulin, and ribonuclease A. Corn proteins eluted at the positions shown: A, native corn proteins V and VI; B, native corn protein IV; C, native corn proteins I, II, and III; D, pyridylethylated corn proteins II, III, IV, V, and VI; E, pyridylethylated β-purothionin; F, pyridylethylated corn protein I.

groups. The first group consisted of fractions I, II, and III, with the largest relative sizes (*K*av = 0.44). Fraction IV was of intermediate size *K*av = 0.47), and fractions V and VI formed a third group with the smallest relative molecular sizes (*K*av = 0.51). In their native condition, all the corn proteins eluted after native β-purothionin; thus, their molecular sizes apparently are slightly smaller than that of the purothionin.

When the corn proteins were reduced and pyridylethylated prior to passage through the Sephadex G-50 column, so that disulfide bonding was disrupted and any tertiary structure due to -S-S- bonds was destroyed, they eluted as if they were much larger than before reduction, and fraction I eluted at K_{av} = 0.20. The reduced and alkylated proteins thus behaved as if they were about 3 times as large after reduction as in their native state. The same phenomenon was found with β-purothionin (*K*av native = 0.41, *K*av reduced and alkylated = 0.21) and is probably due to the fact that all purothionins are apparently tightly folded in the native state (Nimmo et al., 1974) and apparently unfold upon scission of the disulfide bonds. Since the corn proteins showed a similar dramatic size change on reduction, they probably each contained several disulfide bonds that maintained their folded conformations. Since fractions II–VI all eluted at exactly the same volume, they must all be very similar in molecular size—slightly smaller than the fraction I protein. This apparent size difference may also be due to molecular conformation differences caused by something other than disulfide bonds because the amino acid composition data, based on one residue of Thr and Ile per molecule, indicate that proteins II–VI are as large as or larger than protein I.

Amino acid analysis and Sephadex G-50 data thus indicated that all six of the corn proteins are small, basic proteins that are held in a rather tightly folded conformation by disulfide bonds, as are the purothionins.

Toxicity Studies. When each of the six corn proteins was injected into larvae of the tobacco hornworm (*M. Sexta*) at a level of 100 μg per 1 g of larva, none was toxic. A durum wheat β-purothionin sample injected at the same concentration was toxic. Kramer et al. (1979) showed that thionins from various grains all had LD_{50} values of less than 50 μg/g. Either the corn proteins are not toxic to tobacco hornworm larvae or they are at least much less toxic than thionins.

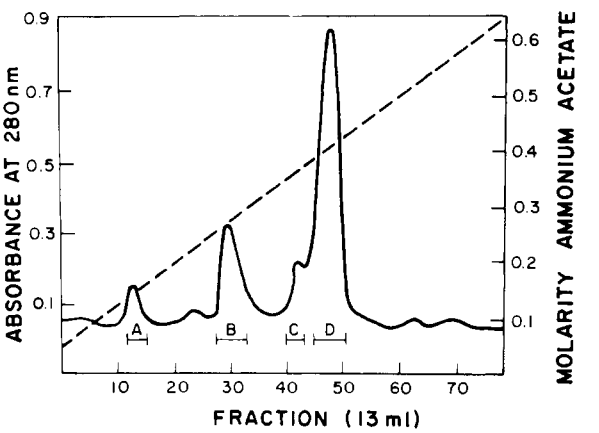

Figure 3. Elution of chymotrypic peptides of corn protein I from a carboxymethylcellulose column with a linear gradient of ammonium acetate. Bars indicate fractions pooled.

Table II. Amino Acid Composition of Chymotryptic Peptides Obtained from Corn Protein I

amino acid	peptide A	peptide B	peptide C	peptide D	whole protein I
Lys	0.8	1.0	1.0	2.1	3.4
His		2.9		2.2	2.8
Arg + Cys[a]	1.4	6.9	3.3	6.7	11.0
Asx		0.9		3.0	3.1
Thr		0.1		0.8	1.0
Ser		1.1		4.2	3.7
Glx	1.3	2.1	1.3	3.7	5.5
Pro		1.0		1.2	1.6
Gly		1.1		4.0	4.1
Ala	0.7		1.0	1.0	2.2
Val				1.6	2.0
Met				1.0	1.2
Ile				0.9	1.0
Leu		2.5		1.0	2.2
Tyr		2.2		0.1	1.7
Phe				1.0	1.0

[a] Arg and (pyridylethyl)cysteine were not separated from each other.

Chymotryptic Peptides. Because the chemical and physical data indicated that corn protein I was similar to rye thionin even though toxicity tests showed that it was biologically different, chymotryptic peptides were prepared and analyzed to determine whether the primary structure of the corn protein I was similar to that of wheat purothionins. All three wheat purothionins (α_1, α_2, and β) yielded two peptides when hydrolyzed with chymotrypsin for 15 min in the native state (Mak and Jones, 1976b). In all cases purothionin hydrolysis occurred adjacent to Tyr (residue 13) and not elsewhere. When native corn protein I was hydrolyzed with chymotrypsin for 15 min, reduced and pyridylethylated, and separated by gradient elution from CM-cellulose, four UV-absorbing peptide fractions were obtained as shown in Figure 3. The 4 fractions were collected and freeze-dried, and their amino acid compositions were determined. The results are shown in Table II. Fractions A and C were small, containing 4 and 6 amino acid residues, respectively. Because all 4 amino acids of A were present in C, it seems probable that A arose from C by removal of 2 Cys or Arg residues. Based on 1 residue each of Phe, Ile, and Thr in whole corn protein I, the protein must contain about 48 residues (exclusive of Trp which was not determined). The Sephadex G-50 data confirmed that the 48-residue length was probably correct. Since peptides B (22 residues) and D (35 residues) together

account for more than the total 48 residues of protein I, the 2 peptides probably overlap each other in the molecule to some extent so that the overlapping residues are counted twice. Alternatively, protein I could consist of 96 amino acids/molecule with 2 residues each of Ile, Phe, and Thr. This is not likely because such a protein, with 2 Phe and 4 Tyr residues, should give rise to 7 chymotryptic peptides instead of the 4 found.

Since purothionins yielded only two peptides when subjected to identical chymotryptic hydrolysis (Mak and Jones, 1976b) and since the purothionin chymotryptic peptides were completely different in amino acid composition from the corn protein chymotrypic peptides, corn protein I probably is not homologous with any of the purothionins.

The corn proteins we isolated are similar to purothionins and to rye thionin in amino acid composition, size, and other chemical and physical characteristics but are not toxic to hornworm larvae. Corn protein I, which was most similar to thionins, apparently is not homologous with purothionins since it yields chymotryptic peptides that are very different from those released from purothionins under similar conditions.

ACKNOWLEDGMENT

We thank Karl J. Kramer for conducting the *M. sexta* toxicity tests, Harriet Meinecke for defatting and extracting the corn, and John Hubbard for conducting the amino acid analyses.

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Received for review December 12, 1979.
Accepted May 12, 1980.

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Amino Acid Content of Baltic Herring and Rainbow Trout Roe

Jukka K. Kaitaranta,* Raila Lamppu, and Reino R. Linko

The amino acid content of Baltic herring and rainbow trout roe at different stages of maturity has been determined. The amino acid pattern with glutamic acid, leucine, aspartic acid, lysine, and alanine as the major components was generally uniform for both roe regardless of the maturity degree. The essential amino acids averaged 35.6 and 37.5 g/100 g of protein in herring and trout roe, respectively, and were in a good balance compared with the FAO/WHO recommendations. The first limiting amino acid in trout roe was tryptophan, and that in herring roe was the total amount of sulfur-containing acids. The amino acid score values were 90.0 and 80.0 for trout and herring roe protein, respectively.

Roe is one of the most valuable food products from fishery sources. When roe is the primary product of fish industry as in the herring industry on the Pacific coast of Canada, the harvesting of roe fish is timed just prior to the spawning to give completely mature roe. If roe is processed as a byproduct, it is collected from normal fish catches during some weeks or months before spawning (e.g., Atlantic and Baltic herring and freshwater whitefish) or during the sacrificing of cultured fish (e.g., rainbow trout). Thus, the degree of maturity of processed roe may vary significantly according to fish species or even in the roe products from the same species manufactured at different times during the roe season.

The nutritive value of roe may change with its maturation. In the gross composition of roe, protein forms the major part of the dry weight (Zaitsev et al., 1969; Vuorela et al., 1979). During maturation of the roe, in addition to the increase in the total protein content, the relative amount of protein may vary, too (Medford and Mackay, 1978; Vuorela et al., 1979). Further, the relative distribution of amino acids has been found to be significantly altered by the maturity of roe of different salmon species (Seagran et al., 1954). The amino acids of mature rainbow trout roe have been reported by Satia et al. (1974) and Cantoni et al. (1975). In certain respects, however, their results differ considerably from each other. In herring roe protein, the constituent amino acids have not been reported in the literature, whereas 10 free amino acids have been identified in Atlantic herring roe (Gjessing, 1963).

The purposes of this study were to determine the amino acid composition of the roe from Baltic herring and rainbow trout, to find out whether or not any significant changes take place during the maturation, and to evaluate the quality of roe protein in relation to the amino acid requirements in human nutrition.

MATERIALS AND METHODS

Fish and Roe. Rainbow trout (*Salmo gairdneri*) from a local fish farm and Baltic herring (*Clupea harengus*) from the Southwest Coast of Finland were obtained at

Technical Research Centre of Finland, Food Research Laboratory, SF-02150 Espoo 15, Finland (J.K.K.), and University of Turku, Department of Chemistry and Biochemistry, SF-20500 Turku 50, Finland (R.L. and R.R.L.).

0021-8561/80/1428-0908$01.00/0