Isolation and Determination of Amino Acid Sequence of Avenothionin, a New Purothionin Analogue from Oat

To the Editor:

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About forty years ago a low molecular weight protein rich in sulfur-containing amino acids and lysine was isolated from wheat by Balls and Hale (1940). Since then this protein, called purothionin, has been thoroughly investigated by many authors and the amino acid sequence of three pure components α_1-, α_2-, and β-purothionin was determined (Jones and Mak 1977, Mak and Jones 1976, Ohtani et al 1975).

Later, purothioninlike proteins (purothionin analogues) were isolated. That from barley (Redman and Fisher 1969) is called

TABLE I
Amino Acid Composition[a] of Avenothionins and Their Differences from α_1-Purothionins and β-Purothionins

Amino Acid	α-Avenothionin Amount	α-Avenothionin Difference[b]	β-Avenothionin Amount	β-Avenothionin Difference
Asp	3.92	+2	4.05	=
Thr	2.06	+1	4.13	+2
Ser	5.02	=	4.42	=
Glu	0.18	−1	0.24	−1
Pro	2.99	+1	3.21	+1
Gly	3.07	−2	2.16	−1
Ala	2.11	−1	2.24	−1
Cys	7.91	=	8.05	=
Val	1.21	=	1.06	=
Ile	1.00	=	0.08	=
Leu	3.92	=	5.00	=
Tyr	0.90	=	0.83	=
Phe	1.07	=	0.94	=
Lys	4.82	=	5.85	=
Arg	4.86	=	4.06	=

[a]Mol of amino acid per mol of protein.
[b]Difference from purothionin value. Values rounded to nearest whole number. Equal sign represents no difference.

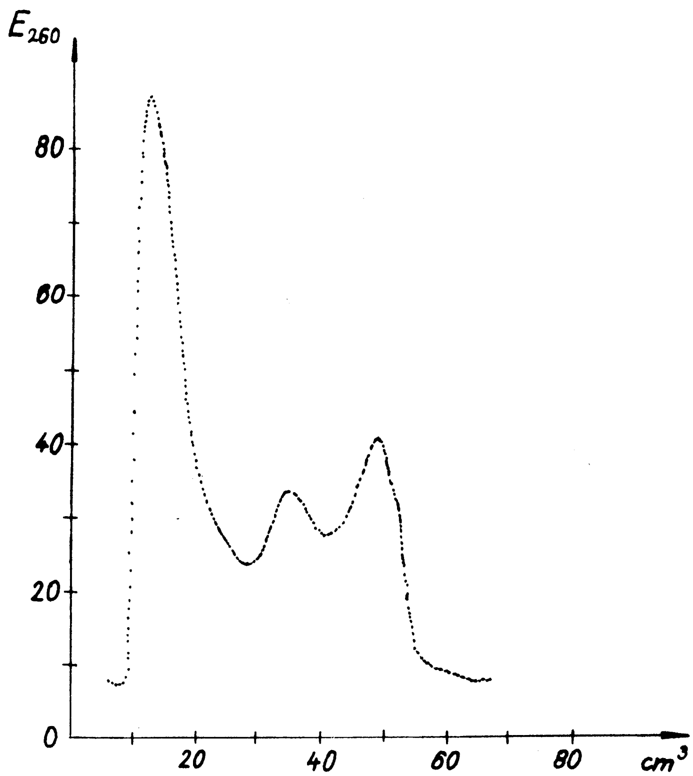

Fig. 1. Elution pattern of avenothionin obtained by Sephadex G 75 column chromatography. The column was 22 × 2 cm. The eluent was 0.01 *M* KCl in 0.05 *M* acetic acid.

TABLE II
Amino Acid Compositions of Some Chymotryptic Avenothionin Peptides and Their Differences from α_1-Purothionin and β-Purothionin Peptides

	Peptide Number/Amino Acid Numbers															
	2/25-33				3/34-55				10/14-24				9/1-8			
	α		β		α		β		α		β		α		β	
Amino Acid	Amount	Difference[a]	Amount	Difference[a]	Amount	Difference	Amount	Difference	Amount	Difference	Amount	Difference	Amount	Difference	Amount	Difference
Asp	0.15	=	0.14	−1	0.92	+1	1.31	=	1.03	=	1.43	=	1.23	+1	1.11	+1
Thr	1.10	+1	1.26	+1	0.36	=	1.14	=	0.21	=	0.08	=	1.04	=	1.34	=
Ser	0.12	=	1.06	+1	3.15	=	2.08	=	1.17	+1	0.21	=	1.07	−1	1.06	−1
Glu	0.18	=	0.34	=	0.21	−1	0.39	=	0.12	−1	0.47	−1	0.21	=	0.27	=
Pro	0.24	=	0.21	=	2.14	=	2.24	=	1.14	+1	1.34	+1	0.17	=	0.34	=
Gly	0.21	−1	0.40	=	1.24	=	1.34	=	1.41	=	1.27	=	0.02	=	0.41	=
Ala	1.22	=	0.34	−1	0.17	=	0.48	=	0.98	−1	2.14	=	0.09	=	0.28	=
Cys	2.91	=	3.06	=	0.94	=	1.14	=	0.79	=	0.87	=	2.18	=	2.17	=
Val	1.07	=	1.14	=	0.31	=	0.47	=	0.14	=	0.34	=	0.10	=	0.37	=
Ile	1.00	=	0.08	=	0.07	=	0.29	=	0.31	=	0.24	=	0.18	=	0.39	=
Leu	0.21	=	1.00	=	1.00	=	1.00	=	2.00	=	2.00	=	1.00	=	1.00	=
Tyr	0.12	=	0.24	=	0.19	=	0.34	=	0.38	=	0.41	=	0.34	=	0.39	=
Phe	0.37	=	0.39	=	1.09	=	1.27	=	0.24	=	0.34	=	0.20	=	0.47	=
Lys	1.17	=	1.14	=	1.96	=	2.14	=	1.10	=	1.11	=	1.18	=	2.08	=
Arg	0.98	=	0.94	=	0.47	=	0.28	=	2.03	=	2.06	=	1.04	=	0.16	=

[a]Difference from purothionin value. Values rounded to nearest whole number. Equal sign represents no difference.

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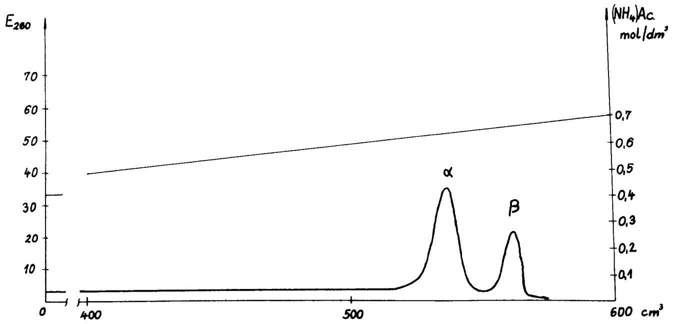

Fig. 2. Elution pattern of α-avenothionin and β-avenothionin from a CM-cellulose column (25 × 2 cm). The sample was eluted with a linear gradient of ammonium acetate, pH 5.2, from 0.4 to 0.7*M*, 300 ml of each.

Table III
Amino Acid Sequences of Thionins

Acid Number	Location in General Sequence	Purothionin α_1	Purothionin α_2	Purothionin β	Avenothionin α	Avenothionin β	Viscotoxin
		Specific Sequences					
X_1	5	Arg	Arg	Lys	Arg	Lys	Pro
X_2	6	Ser	Thr	Ser	Asn	Asn	Asn
X_3	8	Leu	Leu	Leu	Leu	Leu	Thr
X_4	12	Cys	Cys	Cys	Cys	Cys	Ile
X_5	15	Leu	Leu	Leu	Leu	Leu	Ala
X_6	18	Ala	Ser	Ala	Ser	Ala	Leu
X_7	19	Arg	Arg	Arg	Arg	Arg	Thr
X_8	22	Gln	Gln	Gln	Pro	Pro	Pro
X_9	23	Lys	Lys	Lys	Lys	Lys	Arg
X_{10}	24	Leu	Leu	Leu	Leu	Leu	Pro-Thr
X_{11}	26	Ala	Ser	Ala	Ala	Ser	Ala
X_{12}	27	Gly	Thr	Asn	Thr	Thr	Lys
X_{13}	28	Val	Val	Val	Val	Val	Leu
X_{14}	29	Cys	Cys	Cys	Cys	Cys	Ser
X_{15}	30	Arg	Arg	Arg	Arg	Arg	Gly
X_{16}	33	Ile	Leu	Leu	Ile	Leu	Leu
X_{17}	34	Ser	Thr	Thr	Ser	Thr	Ile
X_{18}	37	Leu	Leu	Leu	Leu	Leu	Ser
X_{19}	38	Ser	Ser	Ser	Ser	Ser	Thr
X_{20}	41	Lys	Lys	Lys	Lys	Lys	···
X_{21}	42	Gly	Gly	Asp	Asp	Asp	Ser
X_{22}	43	Phe	Phe	Phe	Phe	Phe	Tyr
X_{23}	44	Pro	Pro	Pro	Pro	Pro	Pro-Asp

General Sequence

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 .
NH_2-Lys-Ser-Cys-Cys-X_1-X_2-Thr-X_3-Gly-Arg-Asn-X_4-Tyr-Asn-X_5-Cys-

17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34
Arg-X_6-X_7-Gly-Ala-X_8-X_9-X_{10}-Cys-X_{11}-X_{12}-X_{13}-X_{14}-X_{15}-Cys-Lys-X_{16}-X_{17}-

35 36 37 38 39 40 41 42 43 44 45
Ser-Gly-X_{18}-X_{19}-Cys-Pro-X_{20}-X_{21}-X_{22}-X_{23}-Lys-COOH

hordothionin and that from rye (Hernandez-Lucas et al) is secalethionin.

Until now we have had no information about isolation of purothionin analogues from oats. In our laboratory we have recently isolated a purothionin analogue from oat called avenothionin and have investigated the properties and structure of this new protein.

The avenothionin was isolated by the method of Balls and Hale (1940). Like purothionin, it was toxic to *Saccharomyces cerevisiae* and *S. uvarum*. The raw avenothionin was fractionated by gel chromatography on Sephadex G 75 (Fig. 1) and investigated by polyacrylamide gel electrophoresis. The low molecular weight components were separated by column chromatography on CMC 52 according to Jones and Mak (1977). Results are shown in Fig. 2. α-Avenothionin could not be separated into further subfractions.

Chymotryptic digestion and fractionation of the chymotryptic peptides was also conducted according to the method of Mak and Jones (1976).

An automatic amino acid analyzer (Tip AAA 881 Microtechna Praha) was used to determine the amino acid composition both of the fractions and of the peptides. The amino acid compositions of the avenothionin fractions and their differences in comparison with α_1-purothionins and β-purothionins are shown in Table I.

On the basis of the large similarity of the primary structures of purothionins and of viscotoxin (Mak and Jones 1976)—purothionin analogues of European mistletoe (*Viscum album* L.)—one can postulate that the thionins have evolutionary conservative sequence sections. Having found the sequences of chymotryptic peptides in the purothionin chains, according to the data of Mak and Jones (1976), and having compared the amino acid compositions of certain peptides of purothionins and avenothionins (Table II), and assuming that conservative peptide sections exist in the avenothionin changes, we could predict the places of the changeable amino acid residues with some probability (Table III).

A more detailed description of the investigations and results is in preparation.

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