

Update section

Sequence

Nucleotide sequence of a cDNA clone that encodes the maize inhibitor of trypsin and activated Hageman factor

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Maize seeds contain a 12 kDa trypsin inhibitor which is also peculiarly selective among plasma proteases in its inhibition of activated Hageman factor [1, 2]. We refer to this protein as CHFI, for 'corn (activated) Hageman factor inhibitor'. We are working to understand the physical basis of the inhibition of activated Hageman factor by CHFI. We have now isolated a cDNA clone that encodes CHFI.

Using as a probe an oligonucleotide whose sequence was based on a portion of the amino acid sequence of CHFI [3], we screened a maize endosperm cDNA library. This cDNA library had been constructed in the BlueScribe vector starting with poly(A)⁺ RNA from dissected endosperm of maize inbred line A188, and was kindly provided by Drs Irwin Rubenstein and John Hunsperger of the University of Minnesota. A cDNA clone (8-1) that apparently encodes the entire protein was isolated. The nucleotide sequence of this DNA and the amino acid sequence inferred from it are shown in Fig. 1. The polypep-

tide encoded by the longest open reading frame contains 155 amino acid residues and has a calculated molecular weight of 16304. The amino acid sequence is identical to the previously determined sequence of CHFI at 94% of the corresponding positions. Two stretches occur in the inferred sequence that do not exist in CHFI as isolated from maize seeds. One of these is the stretch of 28 residues at the N-terminus. This sequence is very similar to sequences of N-terminal signal peptides in other cereal endosperm proteins [4]. The sequence of clone 8-1 also encodes 17 amino acid residues at the C-terminus that were not found in CHFI [3]. It is possible that this region is removed by a post-translational processing mechanism as reported in other seed proteins [5].

Expression studies in yeast have confirmed that the protein product encoded by clone 8-1 is indeed an inhibitor of trypsin and of activated Hageman factor and not of plasma kallikrein (results not shown).

-32	CATCCATCGAGAGGCCGTCGACAGGGAATTA	-1
	ATGGCGTCGTCGTCTAGCAGCAGCCACGCCGCTCATCCTCGCAGCCGCTCCTGCTC	60
	M A S S S S S S H R R L I L A A A V L L	20
	TCCGTGCTCGCGGCTGCCAGCGCCAGCGCGGACCTCCTGCGTGCCGGGTGGGCCATC	120
	S V L A A A S A ↑ S A G T S C V P G W A I	40
	CCGCACAACCCGCTCCCGAGCTGCCGCTGGTACGTGACCAGCCGGACCTGCGGCATCGGG	180
	P H N P L P S C R W Y V T S R T C G I G	60
	CCGCGCCTCCCGTGGCCGGAGCTGAAGAGGAGATGCTGCCGGGAGCTGGCGGACATCCCG	240
	P R L P W P E L K R R C C R E L A D I P	80
	GCGTACTGCCGGTGACCGCGCTGAGCATCCTCATGGACGGCGCGATCCCGCCTGGCCCG	300
	A Y C R C T A L S I L M D G A I P P G P	100
	GACGCGCAGCTGGAGGGCCGCCTAGAGGACCTGCCGGGCTGCCCGGGAGGTGCAGAGG	360
	D A Q L E G R L E D L P G C P R E V Q R	120
	GGATTGCGCGCCACCTCGTCACGGAGGCGAGTGCAACCTGGCCACCATCAGCGGCGTC	420
	G F A A T L V T E A E C N L A T I S G V	140
	GCCGAATGCCCTGGATTCTCGCGCGGGAACGATGCCCTCCAAGTAAGTGCAGAGCA	480
	A E C P W I L G G G T M P S K >	155
	TAGTGCATGAGGAATGAGCTTGTAGCTAGCTCATATGTCTGAATAATAAGCACAGCAAGA	540
	AGATGAATGCATTTCTCGGATCGTTCATCCGGAACAATAATTAAAGGGGATCCGGATTG	600
	TTCTGTGATATAATTAACGATTCTGTATACTTGAAGTAGCTAGGCTCGTCCCCATC	660
	CAATGCAAGCAAAAAAAAAAAAAA	683

Fig. 1. Nucleotide sequence of the cDNA insert in clone 8-1 and the amino acid sequence encoded by the longest open reading frame. The arrow indicates putative site of cleavage of the signal peptide. CHFI isolated from corn seeds has the N-terminal sequence that lies immediately to the right of the arrow.

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