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Isolation and characterization of alliinase cDNA clones from garlic (*Allium sativum* L.) and related species

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cDNA libraries constructed from poly(A)-rich RNA isolated from *Allium sativum* (garlic), *Allium cepa* (onion) and *Allium ascalonicum* (shallot) were screened for cDNA clones encoding the alliinase using colony hybridization. Sequence analysis of the alliinase cDNA clones from different Alliaceae species revealed a high degree of sequence similarity both at the nucleotide and at the amino acid level.

Apparently, the alliinases are translated from mRNA species of approximately 2200 nucleotides. The primary translation products are preproproteins which are converted into the mature alliinases following post-translational modifications. In the case of the garlic alliinase, the mRNA encodes a 486-amino-acid polypeptide with a molecular mass of 55623 Da. Cleavage of the signal peptide (28 amino acids) results in a preprotein which extends 10 amino acids before the first amino acid of the mature protein of 51451 Da. Southern-blot analysis of genomic DNA has shown that the alliinases are most probably encoded by a family of closely related genes, which is in good agreement with the sequence heterogeneity found between different alliinase cDNA clones of one species.

It has been shown previously that most of the characteristic odor of *Allium* species including the vegetables onion (*Allium cepa* L.) and garlic (*Allium sativum* L.) is due to the enzymic degradation of sulfur-containing amino acids (Fowden, 1964). The enzyme involved in this process is the so-called alliinase (or cysteine sulphoxide lyase) which is known to represent an important fraction of the total protein content in *Allium* bulbs and leaves. It is generally believed that the alliinase and the amino acid substrate are present in separated compartments *in vivo*. Upon rupture or wounding of the cells, the enzyme located in the vacuole and the alkyl cysteine sulphoxides located in the cytoplasm of Alliaceae species can react and produce the volatile odorous compounds (Lancaster and Collin, 1981).

The first alliin lyase to be described was the alliinase found in garlic cloves (Stoll and Seebeck, 1951). Since then, there have been numerous reports on the occurrence, purification and characterization of alliinases from several Alliaceae species (Tobkin and Mazelis, 1979; Nock and Mazelis, 1986, 1987; Fujita et al., 1990). From the data available, it seems that the alliinases from different, though related sources differ quite markedly in their pH optimum, K_m values, substrate specificities and molecular structures. Besides *Allium* species, *Acacia* (Mazelis and Creveling, 1975; Sweet and Mazelis, 1987), broccoli (Hamamoto and Mazelis, 1986) and other Brassicaceae species (Mazelis, 1963) were also found to contain alliin lyases in their cells.

The present paper describes the isolation and characterization of cDNA clones encoding the alliinases from *A. sativum*, *A. cepa* and *A. ascalonicum*. Sequence analysis of the complete coding sequence of the different cDNA clones has revealed a high sequence similarity for the alliinases from Alliaceae species.

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Enzyme. Alliinase (EC 4.4.1.4).

Note. The nucleotide sequences reported in this paper have been submitted to the Genbank™/EMBL Data library and are available under accession numbers Z12620, Z12621 and Z12622.

EXPERIMENTAL PROCEDURES

Materials

Bulbs of garlic (*A. sativum* L.), shallot (*A. ascalonicum* L.) and onion (*A. cepa* L.) were purchased from a local store. Oligodeoxythymidine-cellulose was purchased from Sigma Chemical Co. Radioisotopes were obtained from Amersham Corp. A cDNA synthesis kit, the multifunctional phagemid pT_7T_3 18 U, restriction enzymes and DNA-modifying enzymes were obtained from Pharmacia LKB Biotechnology Inc. *Escherichia coli* XL1 Blue competent cells were purchased from Stratagene.

RNA isolation

Total cellular RNA was prepared from plant material stored at −80°C, essentially as described by Finkelstein and Crouch (1986). Poly(A)-rich RNA was enriched by chromatography on oligodeoxythymidine-cellulose as described by Siflow et al. (1979), except that poly(A)-rich RNA was eluted at room temperature.

Purification of the garlic alliinase

The garlic alliinase was purified from peeled garlic cloves essentially as described by Nock and Mazelis (1986). Protein samples were analyzed in a 12.5–25% (mass/vol.) polyacrylamide gradient gel according to Laemmli (1970).

Amino-acid-sequence analysis

Protein sequencing was conducted on an Applied Biosystems model 477A protein sequencer, interfaced with an Applied Biosystems model 120A on-line analyzer.

Construction and screening of cDNA library

cDNA libraries were constructed from total poly(A)-rich mRNA isolated from young and old bulbs, as well as young shoots from garlic and young shoots of shallot and onion using the cDNA synthesis kit from Pharmacia. cDNA fragments were inserted into the *Eco*RI site of the multifunctional phagemid pT_7T_3 18 U. The library was propagated in *E. coli* XL1 Blue.

Recombinant alliinase clones for garlic were screened by colony hybridization using a ^{32}P-end-labeled degenerate oligonucleotide probe (20 nucleotides, 5′-TTC-ATN-GTC-CAN-GTC-ATT/C-TT-3′) derived from residues 1–7 of the N-terminal protein sequence determined for the garlic alliinase. Hybridization was carried out overnight at 42 °C in 0.9 M sodium chloride containing 90 mM Tris/HCl, pH 7.5, 6 mM EDTA, 10 × Denhardt's solution (Denhardt et al., 1978), 0.1% SDS, 180 µg/ml hydrolyzed yeast RNA and 2×10^6 cpm/ml ^{32}P-labeled probe. After hybridization, filters were washed four times in 6 × NaCl/Cit. (1 × NaCl/Cit. = 0.9 M sodium chloride and 0.09 M sodium citrate, pH 7.0) at room temperature for 15 min, followed by a 5-min wash at 42 °C in 6 × NaCl/Cit. Filters were blotted dry, wrapped in Saran Wrap and exposed to Kodak X-Omat S film at −80 °C. In a later stage, the random-primer-labeled garlic alliinase cDNA clone was used as a probe to screen for both onion and shallot alliinase cDNA clones. Hybridization was carried out as described previously (Van Damme et al., 1991). Colonies that produced positive signals were selected and rescreened at low density using the same conditions. Plasmids were isolated from purified single colonies on a miniprep scale using the alkaline-lysis method as described by Mierendorf and Pfeffer (1987) and sequenced by the dideoxy method (Sanger et al., 1977).

Northern blot

RNA electrophoresis was performed according to Maniatis et al. (1982). Approximately 3 µg poly(A)-rich RNA was denatured in glyoxal and Me_2SO and separated in a 1.2% (mass/vol.) agarose gel. Following electrophoresis, the RNA was transferred to Immobilon N membranes (Millipore) and the blot hybridized using a random-primer-labeled alliinase cDNA insert. Blots were prehybridized for 4 h at 42 °C in 50% formamide, 5 × NaCl/P_i/EDTA (1 × NaCl/P_i/EDTA = 0.18 M sodium chloride, 10 mM sodium phosphate, pH 7.4, and 1 mM EDTA), 5 × Denhardt's solution (Denhardt et al., 1978), 10% dextran sulphate and 50 µg/ml denatured salmon sperm DNA and hybridized in the same solution containing 2×10^6 cpm/ml labeled DNA insert from alliinase cDNA clones. Hybridization was performed overnight at 42 °C. Afterwards, blots were washed consecutively in 5 × NaCl/P_i/EDTA (15 min at 42 °C), 1 × NaCl/P_i/EDTA containing 0.1% SDS (30 min at 42 °C) and 0.1 × NaCl/P_i/EDTA containing 0.1% SDS (15 min at room temperature). An RNA ladder (0.16–1.77 kb) and plant ribosomal RNA were used as markers.

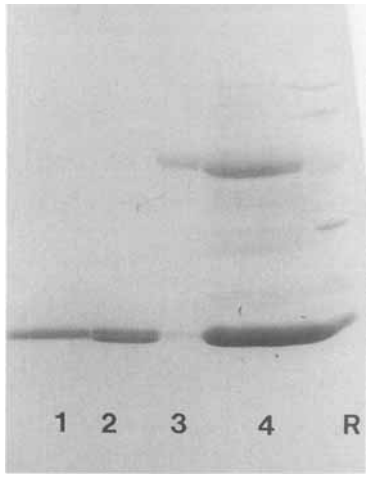

Fig. 1. Gel electrophoresis of garlic lectins ASA II (lane 1) and ASA I (lane 2), garlic alliinase (lane 3) and crude extract from garlic bulbs (lane 4). Lane R shows the M_r reference proteins: (α-lactalbumin, M_r 14400; soya bean trypsin inhibitor, M_r 20100; carbonic anhydrase, M_r 30000; ovalbumin, M_r 43000; bovine serum albumin, M_r 67000; phophorylase b, M_r 94000).

Genomic DNA analysis

Total DNA from garlic was isolated according to the procedures described by Dellaporta et al. (1983) and de Kochko (1990). The DNA preparation was treated with RNAse in order to remove any contaminating RNA. Approximately 30 µg DNA was digested with restriction endonucleases and subjected to electrophoresis in a 0.8% agarose gel. DNA was transferred to Immobilon N membranes (Millipore) and hybridized using the ^{32}P-labeled cDNA insert encoding the garlic alliinase. Hybridization was carried out at 68 °C for 16 h in 6 × NaCl/Cit. containing 0.5% SDS, 5 × Denhardt's solution (Denhardt et al., 1978) and 100 µg/ml denatured salmon sperm DNA. After prehybridization for 4 h, the labeled DNA (2×10^6 cpm/ml) was added to this mixture. Following hybridization, the membrane was washed consecutively in 2 × NaCl/Cit. (30 min at 68 °C), 2 × NaCl/Cit. containing 0.1% SDS (30 min at 68 °C) and 0.5 × NaCl/Cit. (10 min at 68 °C).

Computer analyses

DNA sequences were analyzed using programs from PC Gene and Genepro.

RESULTS

Abundance of the garlic allinase

SDS/polyacrylamide gel electrophoresis of a crude extract from garlic (*A. sativum*) bulbs reveals only two major bands which together account for over 50% of the total protein in this typical vegetative storage tissue (Fig. 1). Whereas the highly abundant low-molecular-mass polypeptides of 11–

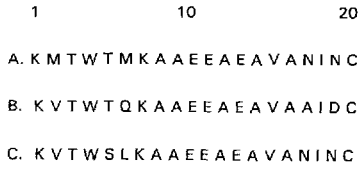

Fig. 2. N-terminal amino acid sequences of alliinases isolated from garlic (A), ramsons (B) and onion (C).

12 kDa have been identified as the composing subunits of the two mannose-specific lectins (Van Damme et al., 1992), the glycopolypeptides with molecular masses around 50 kDa constitute the alliinase described by Nock and Mazelis (1986, 1987). Since it is rather unusual in plants that an enzyme represents one of the major proteins in a storage tissue, the corresponding cDNA clones were isolated and subsequently analyzed.

Isolation and characterization of the garlic alliinase cDNA clone

cDNA libraries constructed from poly(A)-rich RNA, isolated from young garlic bulbs as well as from shoots, were first screened by colony hybridization using a degenerated oligonucleotide probe spanning amino acids 1 – 7 from the N-terminal protein sequence of the alliinase (Fig. 2). Purification of the positive colonies resulting from this screening and subsequent sequencing of the cDNA clones revealed identical sequences for both leaf and bulb alliinase cDNA clones. Upon screening of the total cDNA library using the alliinase cDNA insert as a probe, it became apparent that approximately 1% and 0.5% of the bacterial colonies in the libraries constructed from garlic shoots and young developing bulbs, respectively, contained alliinase sequences. The high number of positive clones is in good agreement with the abundance of the alliinase in garlic leaves and bulbs, and indicates that these young tissues accumulate high concentrations of mRNA encoding the alliinase.

Screening of the garlic cDNA libraries resulted in the isolation of several cDNA clones encoding the complete coding sequence of the garlic alliinase. Sequence analysis of different garlic alliinase clones revealed minor differences in the nucleotide sequences of different clones, indicating that the protein is most probably encoded by a family of closely related genes. Although the libraries were constructed using an oligo-(dT) primer for first strand synthesis, none of the alliinase cDNA clones contained a poly(A) tail.

The nucleotide sequence and the deduced amino acid sequence of the garlic alliinase cDNA clone ALLASA 1 are depicted in Figs 3 and 4. The garlic alliinase clone contains a 1470-bp open reading frame with possible initiation codons at positions 3, 16, 27 and 37 of the deduced amino acid sequence. Translation starting with the first methionine, which according to the criteria of Kozak (1981) is most likely to be used, generates a 486-amino-acid polypeptide with a calculated molecular mass of 55623 Da. Using the rules for protein processing of von Heijne (1986), one possible cleavage site for the processing of the signal peptide was identified between residues 30 and 31. Since cleavage of the signal peptide at this site will generate a polypeptide which starts 10 amino acids before the first amino acid of the mature alliinase (as determined by N-terminal amino acid sequencing of the protein, Fig. 2), we assume that the alliinase is synthesized as a preproprotein. Post-translational cleavage of the propeptide will yield the mature alliinase polypeptide with a calculated molecular mass of 51451 Da (448 amino acids). Four putative glycosylation sites are present in the alliinase sequence at positions 59, 186, 231 and 368. Taken into account the data from Nock and Mazelis (1987), who reported 5.5% carbohydrate for the garlic alliinase, it is most probable that only one of these glycosylation sites is used. The 3′ untranslated region of the cDNA clone ALLASA 1 contains five in-frame stop codons and three putative polyadenylation signals.

As shown in Table 1, the amino acid composition of the mature alliinase as deduced from the nucleotide sequence from the cDNA clone ALLASA 1 and the previously reported amino acid composition of the purified protein (Nock and Mazelis, 1987) are very similar.

Northern-blot analysis

To establish the total length of the RNA encoding the garlic alliinase, a blot containing poly(A)-rich RNA from garlic and related species was hybridized with the labeled alliinase cDNA clone, ALLASA 1. As shown in Fig. 5, hybridization of the blot revealed one band of alliinase mRNA in each lane, indicating the the alliinase gene is not only present in garlic (*A. sativum*) but also in the related species *A. cepa* and *A. ascalonicum*. Using RNA markers, the length of the RNA encoding the alliinases was estimated to be approximately 2200 nucleotides. Identical results were obtained when the blot was hybridized using the labeled alliinase cDNA clone ALLAAA 1 or ALLACA 1.

Furthermore, the expression of alliinase mRNA in various tissues of garlic was examined. It is shown that the garlic alliinase is expressed in shoots as well as in young and old bulbs (Fig. 5). Although the expression level in old bulbs is lower compared to young shoots and bulbs, these results indicate that the alliinase is present throughout development of the plant.

Southern-blot analysis

Since sequence analysis of different garlic alliinase cDNA clones revealed minor differences in their sequences, Southern-blot analysis was performed to establish whether the alliinase is encoded by a gene family. Genomic DNA, isolated from garlic, was digested with different restriction enzymes and electrophoresed in an agarose gel. Hybridization of the blot with the labeled alliinase cDNA clone ALLASA 1 revealed several bands, indicating that the garlic alliinase is indeed encoded by a family of related genes (Fig. 6) since none of the restriction enzymes used cuts the alliinase insert.

Isolation and characterization of alliinase cDNA clones from different Alliaceae species

Since amino acid sequencing of the alliinases isolated from different Alliaceae species revealed a high sequence identity at the N-terminus of the proteins (Fig. 2), the garlic alliinase cDNA clone ALLASA 1 was used as a probe to screen the cDNA libraries constructed from poly(A)-rich RNA isolated from shoots of *A. cepa* and *A. ascalonicum* and isolate the alliinase cDNA clones from the aforementioned species.

The cDNA clone of the shallot alliinase ALLAAA 1 contains a 1343-bp open reading frame which encodes the complete sequence of the mature alliinase and an additional two amino acids, but does not have the information for the signal peptide (Figs 3, 4). Cleavage of these two amino acids will result in a 51062-Da polypeptide (445 amino acids), starting

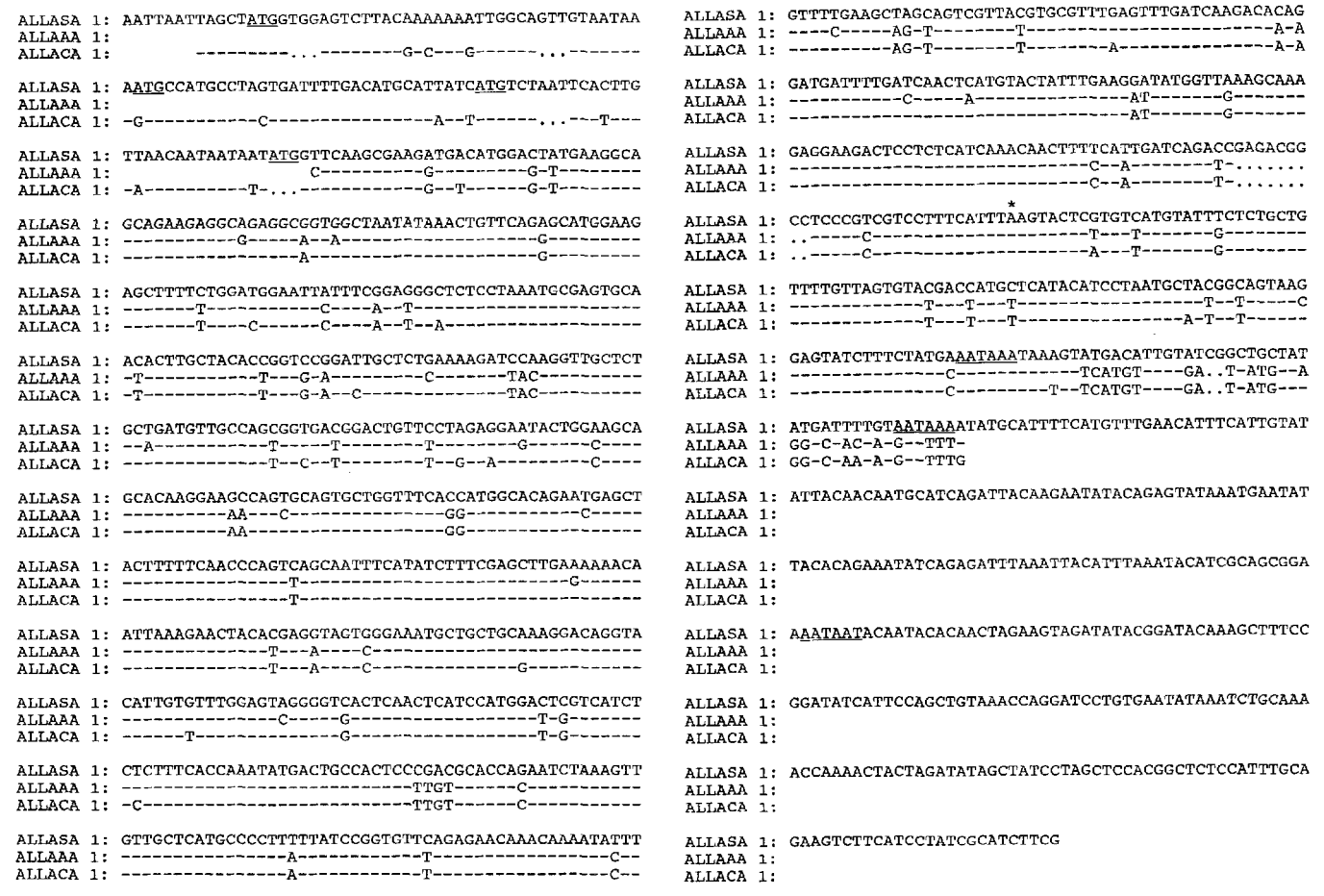

Fig. 3. Comparison of DNA sequences of cDNA clones encoding the alliinase from garlic (ALLASA 1), shallot (ALLAAA 1) and onion (ALLACA 1). The sequences were aligned to maximize similarity. (–) Sequence similarity to ALLASA 1. Dots represent gaps introduced for maximal alignment. Putative start codons and polyadenylation signals are underlined. The stop codon is indicated by an asterisk.

with the first amino acid of the protein. The cDNA clone for the onion alliinase ALLACA 1 encodes a 1442-bp open reading frame which contains the information for the mature alliinase of 51111 Da (445 amino acids) as well as the signal peptide (Figs 3, 4). Like the garlic alliinase, the shallot and onion alliinase contain four potential glycosylation sites at identical positions within the alliinase amino acid sequence. The onion alliinase, however, contains an additional putative glycosylation site at position 308 of its sequence. As shown in Table 1, the amino acid composition of the different alliinases, as deduced from the amino acid sequence of the cDNA clones, is very similar and is in good agreement with the amino acid compositions of the purified alliinases as reported by Nock and Mazelis (1987).

Sequence similarity between alliinase cDNA clones from related species

As shown in Figs 3 and 4, a detailed comparison of the alliinase cDNA clones from different Alliaceae species reveals a high degree of similarity between the different enzymes. However, the alliinase cDNA clones clearly differ from each other at certain positions within their sequence. Maximal alignment of the nucleotide sequences and deduced amino acid sequences from the alliinase cDNA clones isolated from different Alliaceae species was obtained by the introduction

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                                                    ▼
ALLASA 1: laMVESYKKIGSCNKMPCLVILTCIIMSNSLVNNNNMVQAKMTWTMKA
ALLAAA 1:                                          ---V--SL--
ALLACA 1:  --.---D-V--.--V---L--------SF.----I.----VS-SL--

                *
ALLASA 1: AEEAEAVANINCSEHGRAFLDGIISEGSPKCECNTCYTGPDCSEKIQG
ALLAAA 1: ----------G---------L-D----------A---Q--T-
ALLACA 1: ----------G---------L-D----------A------T-

ALLASA 1: CSADVASGDGLFLEEYWKQHKEASAVLVSPWHRMSYFFNPVSNFISFE
ALLAAA 1: ----------------Q----N------G---T--------------
ALLACA 1: ----------------Q----N------G------------------

                                                    *
ALLASA 1: LEKTIKELHEVVGNAAAKDRYIVFGVGVTQLIHGLVISLSPNMTATPD
ALLAAA 1: ----------I-----------------------------------C
ALLACA 1: ----------I-----------------------------------C

                                                  *
ALLASA 1: APESKVVAHAPFYPVFREQTKYFNKKGYVWAGNAANYVNVSNPEQYIE
ALLAAA 1: --Q--------Y----------D----E-K----D---T-T---F--
ALLACA 1: --Q--------Y----------D----E-K----D---T-T---F--

ALLASA 1: MVTSPNNPEGLLRHAVIKGCKSIYDMVYYWPHYTPIKYKADEDILLFT
ALLAAA 1: ----------E---------------------------M---
ALLACA 1: ----------E---------Y-------------------M---

ALLASA 1: MSKFTGHSGSRFGWALIKDESVYNNLLNYMTKNTEGTPRETQLRSLKV
ALLAAA 1: ---Y--------------------K-----------S---------I
ALLACA 1: ---Y----------------T---K-----------S---------I

                                                  *
ALLASA 1: LKEVVAMVKTQKGTMMDLNTFGFKKLRERWVNITALLDQSDRFSYQEL
ALLAAA 1: ----T--I---------------Q-------------K-------K-
ALLACA 1: ----I------N---R----------------------K-------K-

ALLASA 1: PQSEYCNYFRRMRPPSPSYAWVKCEWEEDKDCYQTFQNGRINTQNGVG
ALLAAA 1: ----------------------------------------------S-E-
ALLACA 1: ----------------------------------------------S-E-

ALLASA 1: FEASSRYVRLSLIKTQDDFDQLMYYLKDMVKAKRKTPLIKQLFIDQTE
ALLAAA 1: ---G-----------K-----------I--E-----------SN--I.
ALLACA 1: ---G-----------K-----------I--E-----------SN--I.

ALLASA 1: TASRRPFI
ALLAAA 1: ..------
ALLACA 1: ..------
```

Fig. 4. Comparison of deduced amino acid sequences of cDNA clones encoding the alliinase from garlic (ALLASA 1), shallot (ALLAAA 1) and onion (ALLACA 1). The arrowhead indicates the possible processing site for the cleavage of the signal peptide. (–) Sequence similarity to ALLASA 1. Putative glycosylation sites are indicated by an asterisk. Since the first ATG codon is most probably used as the translation initiation site, the deduced amino acids preceding this methionine are shown in lower case letters.

of some deletions in the sequences of the shallot and the onion alliinase when compared to the garlic alliinase. Apparently the mature garlic alliinase contains three additional amino acids near the C-terminus of the protein. A detailed comparison of the signal peptides and N-terminal propeptides for the onion and the garlic alliinase also reveals some striking differences in the sequences.

The sequence similarity for the total coding region was calculated to be approximately 88% for the garlic and the shallot alliinase, and the garlic and the onion alliinase, whereas the shallot and the onion alliinase showed about 98% similarity at the amino acid level.

Despite the differences in amino acid sequences, all alliinase clones isolated encode proteins with similar amino acid compositions (Table 1). Furthermore, similar isoelectric points were calculated for the different alliinases being 7.94 for the mature garlic alliinase, 8.33 for the shallot alliinase and 8.20 for the onion alliinase. The conservation of the basic structure among these enzymes is further illustrated by computer analysis of the hydropathic plots of the mature alliinases which clearly show the similarity in the distribution of the hydrophilic and hydrophobic regions in their amino acid sequences and predicted secondary structures of the enzymes (results not shown).

Table 1. Amino acid composition of alliinase cDNA clones compared to the protein. The data for the garlic and onion proteins are taken from Nock and Mazelis (1987).

Amino acid	Amino acid composition of				
	garlic		onion		shallot
	ALLASA 1	protein	ALLACA 1	protein	ALLAAA1
Asx	9.8	11.6	9.5	11.1	9.4
Thr	6.0	6.8	6.1	5.5	6.7
Ser	6.9	7.3	7.1	7.7	7.4
Glx	10.9	11.0	10.8	9.5	10.7
Pro	4.7	4.3	4.1	4.2	4.0
Gly	5.3	7.0	6.1	8.2	6.1
Ala	6.5	6.9	5.9	6.0	5.8
Val	6.7	6.4	5.6	6.9	5.4
Cys	2.2	1.6	2.5	0.4	2.5
Met	2.9	0.6	2.7	0.6	2.5
Ile	4.7	3.4	5.2	4.8	5.4
Leu	6.7	6.8	7.0	6.9	7.0
Tyr	5.3	5.5	5.9	5.2	5.6
Phe	4.9	4.3	4.5	4.4	4.5
Lys	7.6	7.2	7.9	6.0	8.1
His	2.0	2.0	2.0	7.0	2.0
Arg	4.7	4.9	4.7	4.4	4.7
Trp	2.0	2.2	2.0	1.2	2.0

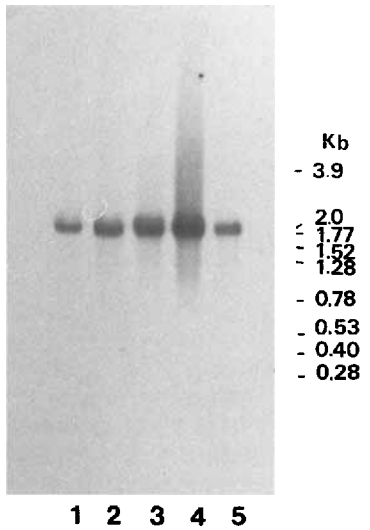

Fig. 5. Northern-blot of poly(A)-rich RNA isolated from different Alliaceae species. Poly(A)-rich RNA was isolated from shoots of shallot (lane 1), onion (lane 2) and garlic (lane 3) and young (lane 4) and old (lane 5) garlic bulbs. The blot was hybridized using the ^{32}P-labeled cDNA insert from ALLASA 1.

DISCUSSION

In this paper, we present the amino acid sequence of the garlic alliinase deduced from a cDNA clone designated ALLASA 1. This cDNA clone encodes a preproprotein of 486 amino acids with a calculated molecular mass of 55623 Da which is translated from an mRNA of approximately 2.2 kb. Since the alliinase was reported to be located in the vacuole (Lancaster and Collin, 1981), we searched for the presence of a signal peptide. Using the rules for protein processing of von Heijne (1986), a cleavage site for the signal peptide was identified indeed between residues 30 and 31 of the prepropro-

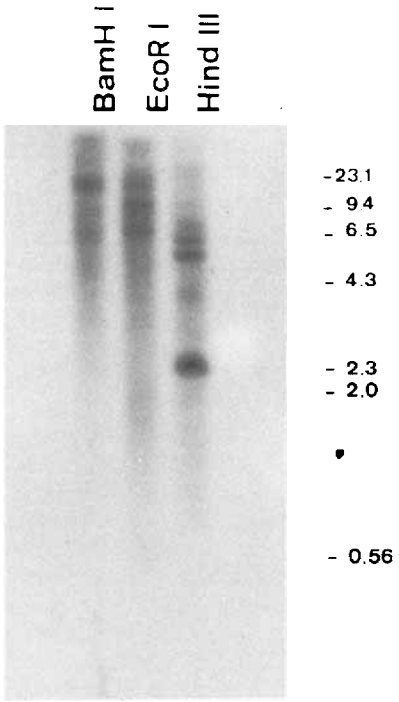

Fig. 6. Southern blot of genomic DNA isolated from garlic bulbs. DNA was digested with *Bam*HI, *Eco*RI and *Hin*dIII, and hybridized with the ^{32}P-labeled cDNA insert from ALLASA 1. Numbers on the right show DNA size (kb).

tein. However, since processing of the signal peptide at this site results in a polypeptide which extends 10 amino acids before the first amino acid of the mature alliinase as determined by N-terminal amino acid sequencing of the protein, the alliinase proprotein will undergo a post-translational modification during maturation of the enzyme. N-terminal pro-domains have been reported before for the vacuolar storage proteins napin (Ericson et al., 1986) and sporamin (Hattori et al., 1987), where they have been found to contain the vacuolar sorting information. Although at present several peptide sequences have been shown to be involved in the vacuolar targeting of proteins, no consensus sequence has been identified. Moreover, a comparison of these sequences does not allow one to define a general vacuolar targeting motif although the presence of hydrophobic amino acids is probably a common feature, particularly for C-terminal propeptides (Chrispeels and Raikhel, 1992; Vitale and Chrispeels, 1992). Comparison of the propeptide of the alliinase precursor did not show any sequence similarity to the previously reported pro-domains from napin and sporamin.

The results reported in the literature contain several discrepancies concerning the molecular structure and physical properties of the alliinases. Most of this confusion is probably due to the fact that the enzyme is very difficult to purify to homogeneity and tends to aggregate and precipitate when it is kept in aqueous solution. Subunit molecular masses ranging over 47–65 kDa have been reported for the garlic enzyme. Our results of the molecular cloning of the alliinase have now shown that the cDNA clone encodes a polypeptide with a calculated molecular mass of 51451 Da for the mature alliinase. Taking into account that the garlic alliinase is a glycoprotein containing 5.5% carbohydrate (Nock and Mazelis, 1987), we assume that on average only one of the four potential glycosylation sites in the alliinase sequence is used, resulting in a subunit molecular mass around 55 kDa.

Sequence analysis of several cDNA clones encoding the garlic alliinase revealed some sequence heterogeneity between the different clones. This is in good agreement with the charge heterogeneity observed by Nock and Mazelis (1987) on isoelectric focusing and pointed to the existence of a gene family. The occurrence of multiple genes encoding the alliinase was confirmed by Southern-blot analysis of genomic DNA.

Using the garlic alliinase cDNA clone as a probe, we isolated two very homologous alliinase cDNA clones from cDNA libraries constructed from poly(A)-rich RNA isolated from shoots of *A. cepa* and *A. ascalonicum*. The overall sequence similarity for the coding region of the alliinases was at least 88% at the amino acid level. These results are in contrast with the data from Nock and Mazelis (1989), who reported very little structural similarity between the alliinases from onion and garlic. However, the molecular cloning of the enzymes shows that, despite the differences in their sequences, all cDNA clones encode alliinases with very similar amino acid compositions and isoelectric points. Furthermore, computer analysis of the hydropathic plots and predicted secondary structure for the alliinases suggested that the enzymes have similar polypeptide folding patterns.

Since computer searches in the EMBL DNA sequence databases revealed no significant sequence similarity of the alliinase cDNA sequences to any other known sequence, no comparisons can be made to any other protein.

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REFERENCES

Chrispeels, M. J. & Raikhel, N. V. (1992) *Cell* 68, 613–616.
de Kochko, A. & Hamon, S. (1990) *Plant Mol. Biol. Rep.* 8, 3–7.
Dellaporta, S. L., Wood, J. & Hicks, J. B. (1983) *Plant Mol. Biol. Rep.* 1, 19–21.
Denhardt, D. J., Dressler, D. & Ray, D. S. (1978) *The single-stranded DNA phages*, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, USA.
Ericson, M. L., Rödin, J., Lenman, M., Glimelius, K., Josefsson, L.-G. & Rask, L. (1986) *J. Biol. Chem.* 261, 14576–14581.
Finkelstein, R. R. & Crouch, M. L. (1986) *Plant Physiol.* 81, 907–912.
Fowden, L. (1964) *Annu. Rev. Biochem.* 33, 173–204.
Fujita, M., Endo, M. & Sano, M. (1990) *Agric. Biol. Chem.* 54, 1077–1079.
Hamamoto, A. & Mazelis, M. (1986) *Plant Physiol.* 80, 702–706.
Hattori, T., Ichihara, S. & Nakamura, K. (1987) *Eur. J. Biochem.* 166, 533–538.
Kozak, M. (1981) *Nucleic Acids Res.* 9, 5233–5252.
Laemmli, U. K. (1970) *Nature* 227, 680–685.
Lancaster, J. E. & Collin, H. A. (1981) *Plant Sci. Lett.* 22, 169–176.
Maniatis, T., Fritsch, E. F. & Sambrook, J. (1982) *Molecular cloning: a laboratory manual*, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, USA.
Mazelis, M. (1963) *Phytochemistry (Oxf.)* 2, 15–22.
Mazelis, M. & Creveling, R. K. (1975) *Biochem. J.* 147, 485–491.
Mierendorf, R. C. & Pfeffer, D. (1987) *Methods Enzymol.* 152, 556–562.
Nock, P. L. & Mazelis, M. (1986) *Arch. Biochem. Biophys.* 249, 27–33.

Nock, P. L. & Mazelis, M. (1987) *Plant Physiol. 85*, 1079–1083.
Nock, P. L & Mazelis, M. (1989) *Phytochemistry (Oxf.) 28*, 729–731.
Sanger, F., Nicklen, S. & Coulson, A. R. (1977) *Proc. Natl Acad. Sci. USA 74*, 5463–5467.
Siflow, C. D., Hammett, J. R. & Key, J. L. (1979) *Biochemistry 18*, 2725–2731.
Stoll, A. & Seebeck, E. (1951) *Adv. Enzymol. 11*, 377–400.
Sweet, W. J. & Mazelis, M. (1987) *Phytochemistry (Oxf.) 26*, 945–948.
Tobkin, H. E. Jr. & Mazelis, M. (1979) *Arch. Biochem. Biophys. 193*, 150–157.
Van Damme, E. J. M., Declercq, N., Claessens, F., Hemschoote, K., Peeters, B. & Peumans, W. J. (1991) *Planta (Berl.) 186*, 35–43.
Van Damme, E. J. M., Smeets, K., Torrekens, S., Van Leuven, F., Goldstein, I. J. & Peumans, W. J. (1992) *Eur. J. Biochem. 206*, 413–420.
Vitale, A. & Chrispeels, M. J. (1992) *BioEssays 14*, 151–160.
von Heijne, G. (1986) *Nucleic Acids Res. 11*, 4683–4690.