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# Cloning and nucleotide sequence of cDNA for the plastid glycerol-3-phosphate acyltransferase from squash

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The partial amino acid sequence and amino acid composition of acyl-(acyl-carrier-protein):glycerol-3-phosphate acyltransferase purified from squash cotyledons were determined. cDNAs encoding this enzyme were isolated from λgt11 cDNA libraries made from poly(A)$^+$ RNA of squash cotyledons by immunological selection and cross-hybridization. One of the resultant clones contained a cDNA insert of 1426 base pairs and an open reading frame of 1188 base pairs. The amino acid sequence deduced from the nucleotide sequence matched the partial amino acid sequence determined for the enzyme. The results suggest that a precursor protein of 396 amino acid residues is processed to the mature enzyme of 368 amino acid residues, losing a leader peptide of 28 amino acid residues. Relative molecular masses of the precursor and mature proteins were calculated to be 43838 and 40929 Da, respectively.

cDNA cloning; Chilling sensitivity; Chloroplast enzyme; Glycerol-3-phosphate acyltransferase; Lipid synthesis; (Squash)

## 1. INTRODUCTION

Acyl-(acyl-carrier-protein):glycerol-3-phosphate acyltransferase (EC 2.3.1.15), designated as glycerol-P acyltransferase in higher-plant plastids, transfers the acyl group from acyl-(acyl-carrier protein) to the C-1 position of glycerol 3-phosphate to synthesize lysophosphatidic acid [1]. This reaction is the first step in the biosynthesis of phosphatidylglycerol in both 18:3 and 16:3 plants [2,3] and of glycolipids in 16:3 plants [4,5]. It has been hypothesized that the substrate selectivity of this enzyme determines the proportion of the dipalmitoyl- plus 1-palmitoyl-2-(*trans*-3)hexadecenoyl molecular species of phosphatidylglycerol in chloroplast membranes [6], which has been shown to be correlated with the chilling sensitivity of higher plants [7–9].

In a previous study [10], three isomeric forms of chloroplast glycerol-P acyltransferase, denoted AT1–AT3, were found in chloroplasts of squash, a chilling-sensitive plant. AT2 and AT3 were purified to single components. The same enzyme has been partially purified from chilling-resistant plants, spinach and pea [11]. Substrate selectivity examined for the squash isomeric forms [12] and for enzymes from spinach and pea [1] is consistent with the hypothetical role of glycerol-P acyltransferase in determining the phosphatidylglycerol molecular species [6].

We report here the isolation and nucleotide sequence determination of a cDNA cloned for glycerol-P acyltransferase from squash cotyledons.

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*Abbreviations:* AT1–AT3, isomeric forms of squash glycerol-P acyltransferase; glycerol-P acyltransferase, acyl-(acyl-carrier-protein):glycerol-3-phosphate acyltransferase

The nucleotide sequence presented here has been submitted to the EMBL/GenBank database under the accession no. Y00771

## 2. MATERIALS AND METHODS

### 2.1. *Plant material, enzyme purification and antibody preparation*

Greening cotyledons of a squash, *Cucurbita moschata* Duch cv. Shirakikuza, were obtained as in [10]. Two isomeric forms



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of squash glycerol-P acyltransferase, AT2 and AT3, were purified to single components according to [10]. The purified AT3 (5 μg each) was injected 10 times into peritonea of mice every week. The resultant antisera reacted with both AT2 and AT3.

2.2. *Amino acid sequence and amino acid composition*

Purified AT2 and AT3, each amounting to about 8 μg, in 0.1 ml of 10 mM Tris-HCl (pH 6.0 at 25°C)/2 mM dithiothreitol/160 mM NaCl/15% (v/v) glycerol were dialyzed twice vs 1 l of 0.02% (w/v) SDS/10 mM $NH_4HCO_3$ after addition of 0.1 mg SDS/5 μl 2-mercaptoethanol. This step was necessary to remove Tris base and glycerol, which could disturb the following amino acid sequence analysis. The dialyzed solution was lyophilized, and the resultant residue dissolved in 0.03 ml $H_2O$ and applied to a protein sequence analyzer (model 470A, Applied Biosystems) equipped with a liquid chromatograph (PTH analyzer, model 120A, Applied Biosystems). The lyophilized AT2 and AT3, each amounting to about 8 μg, prepared as above were also subjected to fragmentation with CNBr according to Koide and Ikenaka [13] except that the molar ratio of CNBr to protein was 5000. The resultant polypeptides were analyzed with a gel-electrophoresis system (Phast System, Pharmacia) for determination of their relative molecular masses and amino acid sequences according to Matsudaira [14].

For analysis of amino acid composition, AT2 and AT3, each amounting to about 20 μg, dissolved in 1.5 ml of 10 mM Tris-HCl (pH 6.0 at 25°C)/2 mM dithiothreitol/160 mM NaCl/15% (v/v) glycerol were applied to a reverse-phase column (ProRPC HR5/10, Pharmacia), which had been equilibrated with 0.1% (w/v) trifluoroacetic acid in $H_2O$. They were eluted with 18 ml of a 0–100% (v/v) linear gradient of acetonitrile/$H_2O$ containing 0.1% (w/v) trifluoroacetic acid. Both AT2 and AT3 were eluted at about 50% acetonitrile/$H_2O$. They were lyophilized and then hydrolyzed at 110°C for 24 h in gaseous HCl according to Bidlingmeyer et al. [15]. The amino acid composition was determined on an amino acid analyzer (model 835, Hitachi).

2.3. *Construction and screening of the cDNA library*

Total RNA was extracted from greening squash cotyledons by the guanidinium thiocyanate method [16], and poly(A)$^+$ RNA purified from the extract by chromatography on oligo(dT)-cellulose [17]. A λgt11 random-primed cDNA library and a λgt11 oligo(dT)-primed cDNA library of $2 \times 10^6$ recombinants were both constructed from the poly(A)$^+$ RNA according to Huynh et al. [18], except that the second strand of cDNA was synthesized by nick translation [19].

The random-primed cDNA library was screened with the antisera raised against AT3 according to Huynh et al. [18], except that positive plaques were detected by the anti-mouse IgG horse IgG/horseradish peroxidase system (vectastain ABC kit, Vector Laboratory). Six positive clones were obtained by this screening. For the purpose of epitope selection [20], antibodies, which specifically bound to the translation products of these clones, were purified from the antisera as described by Weinberger et al. [20], except that phages were plated at a density of $2 \times 10^4$ plaque-forming units per plate, antibodies were washed out from a nitrocellulose membrane with a medium omitting Triton X-100 and the Western blot analysis was performed as in [21]. The antibodies purified with one of the six clones reacted with AT2 and AT3, and this clone designated as λAT01 was used for further study. However, none of the antibodies purified with the other five clones reacted with either AT2 or AT3.

An [$\alpha$-$^{32}$P]dCTP-labeled probe was prepared from the cDNA insert of λAT01 by the random-primer extension method [22]. The cDNA libraries were screened with this probe according to Maniatis et al. [17], except that bovine serum albumin was omitted from the hybridization solution. Two clones, designated as λAT02 and λAT03, were isolated by this screening.

2.4. *Analysis of the nucleotide sequence*

The strategy used for the sequence analysis of cDNA is summarized in fig.1. The cDNA inserts of λ phages were subcloned into the *Eco*RI site of a plasmid pTZ18R (Pharmacia), and the recombinant plasmids were amplified in *Escherichia coli* JM105. The amplified cDNAs were recloned into an appropriate restriction site of pTZ18R. The nucleotide sequence of the recloned cDNA was determined by the dideoxy chain-termination method [23]. Primers for nucleotide chain elongation were prepared with a DNA synthesizer (model 381A, Applied Biosystems).

2.5. *Blot hybridization analysis of mRNA*

Blot hybridization analysis of mRNA followed the procedure of Maniatis et al. [17]. Poly(A)$^+$ RNA was subjected to electrophoresis on 1.0% agarose gel and then transferred to GeneScreen Plus membrane (Dupont). The membrane was preincubated at 65°C for 5 h in a solution containing 5 × Denhardt's solution (1 × Denhardt's solution: 0.02% Ficoll/0.02% polyvinylpyrrolidone)/5 × SSC (1 × SSC: 0.15 M NaCl/15 mM trisodium citrate; pH 7.0)/10% (w/v) dextran sulfate/1.0% SDS/0.01% (w/v) salmon sperm DNA. An [$\alpha$-$^{32}$P]dCTP-labeled probe prepared from the cDNA insert of λAT03 by the random-primer extension method [22] was added to the preincubated solution, and hybridization was performed at 65°C overnight. The membrane was rinsed at room temperature twice with 2 × SSC/0.1% SDS, then three times with 0.1 × SSC/0.1% SDS.

## 3. RESULTS

### 3.1. *Amino acid sequence and amino acid composition*

The amino acid sequence at the amino terminus of AT2 was X 1-Pro 2-Ala 3-His 4-Ser 5-Arg 6-Lys 7-Phe 8-Leu 9-Asp 10-Val 11-Arg 12-Ser 13-Glu 14-Glu 15-Glu 16-Leu 17-Leu 18 (X representing an amino acid which was present, but which could not be identified). Both AT2 and AT3 were fragmented with CNBr to yield polypeptides of relative molecular mass about 18 kDa. The amino acid sequence at the amino terminus of the 18 kDa fragment from AT2 was X 1-X 2-Leu 3-Tyr 4-Gln 5-Asn 6-Tyr 7-Arg 8-Asn 9-Ala 10-Val 11-Ile

Table 1

Amino acid composition determined for two isomeric forms of glycerol-P acyltransferase, AT2 and AT3, from squash, and that calculated from the amino acid sequence deduced from the cDNA nucleotide sequence

| Amino acid | AT2 | AT3 | Deduced from cDNA |
|---|---|---|---|
| Gly | 22 | 24 | 21 |
| Ala | 31 | 32 | 30 |
| Val | 22 | 21 | 22 |
| Leu | 37 | 37 | 36 |
| Ile | 24 | 25 | 27 |
| Phe | 16 | 16 | 16 |
| Ser | 25 | 25 | 29 |
| Thr | 13 | 12 | 12 |
| Tyr | 11 | 10 | 10 |
| Met | 7 | 6 | 7 |
| Cys | nd | nd | 4 |
| Lys | 20 | 20 | 20 |
| His | 10 | 10 | 10 |
| Pro | 25 | 25 | 25 |
| Arg | 18 | 18 | 17 |
| Trp | nd | nd | 3 |
| Asx | 39 | 39 | 38 |
| Glx | 41 | 41 | 41 |
| Total | 361 | 361 | 368 |

nd, not determined

12-Glu 13-Ser 14-Gly 15-Asn 16-Pro 17-Lys 18-Ala 19-X 20-X 21-Ile 22-Val 23. The amino-terminal sequence of the 18 kDa fragment from AT3 determined for 14 amino acid residues was identical with the corresponding sequence of the 18 kDa fragment from AT2.

Table 1 compares the amino acid compositions of AT2 and AT3. They were almost identical, the minor difference being within experimental error.

### 3.2. *Isolation of cDNA clones*

Six positive clones were selected by screening $5 \times 10^5$ plaques from the random-primed cDNA library with the antisera raised against AT3. They were further subjected to epitope selection to yield one positive clone, designated λAT01, containing a

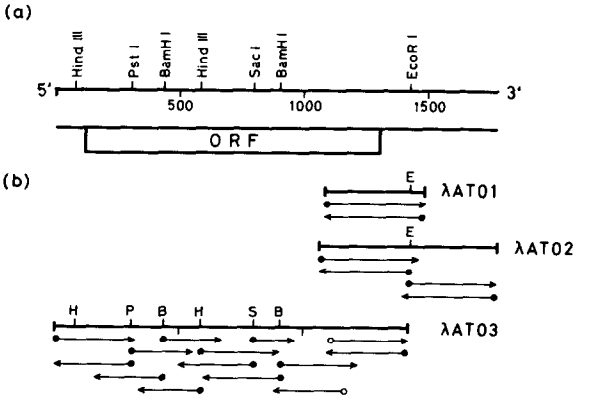


Fig.1. (a) Restriction map of cDNA for the plastid glycerol-3-phosphate acyltransferase from squash. ORF, open reading frame. (b) Strategy for determination of nucleotide sequence. Arrows designate the direction and extent of the nucleotide sequence determined for each fragment. (●) Universal primers and (○) unique primers (17 nucleotides) used for sequence determination of fragments.

cDNA insert of 400 bp. About $1 \times 10^6$ plaques derived from the oligo(dT)-primed cDNA library were screened with the cDNA insert of λAT01 and yielded one positive clone. This clone, designated λAT02, contained a cDNA insert of 718 bp with the poly(A) tracks of about 40 bp. Finally, $5 \times 10^5$ plaques from the random-primed cDNA library were screened with the cDNA insert of λAT01 and yielded 14 positive clones. One of these clones, designated as λAT03, contained the longest cDNA insert of 1426 bp and an open reading frame of 1188 bp. The nucleotide sequence determination indicated that the cDNA inserts of the three clones overlapped each other (fig.1).

### 3.3. *Nucleotide sequence and deduced amino acid sequence*

The nucleotide sequence of a 1781-bp cDNA containing a glycerol-P acyltransferase gene was constructed from the cDNA inserts of λAT02 and λAT03, and its amino acid sequence was deduced from the coding sequence of 1188 bp which cor-

Fig.2. cDNA nucleotide sequence and deduced amino acid sequence of the squash glycerol-P acyltransferase. The deduced amino acid sequence is numbered beginning from 1 for the first amino acid of the mature protein. The predicted leader peptide is indicated by negative numbers. An arrow indicates the predicted processing site. AM denotes the stop codon. Underlined portions of the amino acid sequence matched the partial amino acid sequences determined for AT2. Dashed-underline portions indicate the amino acid residues which were present in AT2 and AT3 but not identified. The double-underlined portion may correspond to the signal for polyadenylation.

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   1 GCCTTCTCTAGGGTTTCTCTTCCTCCCTCCTCCTCTTCTCTCAAGCTCCTTCCCCTTTCTCTGCAATTCGGACCTCCCA
  80 AGCTTGCCTCCTCGTGCTCGCTTCGGTTTTCCGCTTCCAGAGCA

 124 ATG GCG GAG CTT ATC CAG GAT AAG GAG TCC GCC CAG AGT GCT GCC ACC GCT GCT GCT GCT
 -28 Met Ala Glu Leu Ile Gln Asp Lys Glu Ser Ala Gln Ser Ala Ala Thr Ala Ala Ala Ala

 184 AGC TCC GGT TAT GAA AGA CGG AAT GAG CCG GCT CAC TCC CGC AAA TTT CTC GAT GTT CGC
  -8 Ser Ser Gly Tyr Glu Arg Arg Asn Glu Pro Ala His Ser Arg Lys Phe Leu Asp Val Arg

 244 TCT GAA GAA GAG TTG CTC TCC TGC ATC AAG AAG GAA ACA GAA GCT GGA AAG CTG CCT CCA
  13 Ser Glu Glu Glu Leu Leu Ser Cys Ile Lys Lys Glu Thr Glu Ala Gly Lys Leu Pro Pro

 304 AAT GTT GCT GCA GGA ATG GAA GAA TTG TAT CAG AAT TAT AGA AAT GCT GTT ATT GAG AGT
  33 Asn Val Ala Ala Gly Met Glu Glu Leu Tyr Gln Asn Tyr Arg Asn Ala Val Ile Glu Ser

 364 GGA AAT CCA AAG GCA GAT GAA ATT GTT CTG TCT AAC ATG ACT GTT GCA TTA GAT CGC ATA
  53 Gly Asn Pro Lys Ala Asp Glu Ile Val Leu Ser Asn Met Thr Val Ala Leu Asp Arg Ile

 424 TTG TTG GAT GTG GAG GAT CCT TTT GTC TTC TCA TCA CAC CAC AAA GCA ATT CGA GAG CCT
  73 Leu Leu Asp Val Glu Asp Pro Phe Val Phe Ser Ser His His Lys Ala Ile Arg Glu Pro

 484 TTT GAT TAC TAC ATT TTT GGC CAG AAC TAT ATA CGG CCA TTG ATT GAT TTT GGA AAT TCA
  93 Phe Asp Tyr Tyr Ile Phe Gly Gln Asn Tyr Ile Arg Pro Leu Ile Asp Phe Gly Asn Ser

 544 TTC GTT GGT AAC CTT TCT CTT TTC AAG GAT ATA GAA GAG AAG CTT CAG CAG GGT CAC AAT
 113 Phe Val Gly Asn Leu Ser Leu Phe Lys Asp Ile Glu Glu Lys Leu Gln Gln Gly His Asn

 604 GTT GTC TTG ATA TCA AAT CAT CAG ACT GAA GCA GAT CCA GCT ATC ATT TCA TTG TTG CTT
 133 Val Val Leu Ile Ser Asn His Gln Thr Glu Ala Asp Pro Ala Ile Ile Ser Leu Leu Leu

 664 GAA AAG ACA AAC CCA TAT ATT GCA GAA AAC ACG ATC TTT GTG GCA GGG GAT AGA GTT CTT
 153 Glu Lys Thr Asn Pro Tyr Ile Ala Glu Asn Thr Ile Phe Val Ala Gly Asp Arg Val Leu

 724 GCA GAC CCT CTT TGC AAG CCC TTC AGC ATT GGA AGG AAT CTT ATT TGT GTT TAT TCA AAA
 173 Ala Asp Pro Leu Cys Lys Pro Phe Ser Ile Gly Arg Asn Leu Ile Cys Val Tyr Ser Lys

 784 AAG CAC ATG TTC GAT ATT CCT GAG CTC ACA GAA ACA AAA AGG AAA GCA AAC ACA CGA AGT
 193 Lys His Met Phe Asp Ile Pro Glu Leu Thr Glu Thr Lys Arg Lys Ala Asn Thr Arg Ser

 844 CTT AAG GAG ATG GCT TTA CTC TTA AGA GGT GGA TCA CAA CTA ATA TGG ATT GCA CCC AGT
 213 Leu Lys Glu Met Ala Leu Leu Leu Arg Gly Gly Ser Gln Leu Ile Trp Ile Ala Pro Ser

 904 GGT GGT AGG GAC CGG CCG GAT CCC TCG ACT GGA GAA TGG TAC CCA GCA CCC TTT GAT GCT
 233 Gly Gly Arg Asp Arg Pro Asp Pro Ser Thr Gly Glu Trp Tyr Pro Ala Pro Phe Asp Ala

 964 TCT TCA GTG GAC AAC ATG AGA AGG CTT ATT CAA CAT TCG GAT GTT CCT GGG CAT TTG TTT
 253 Ser Ser Val Asp Asn Met Arg Arg Leu Ile Gln His Ser Asp Val Pro Gly His Leu Phe

1024 CCC CTT GCT TTA TTA TGT CAT GAC ATC ATG CCC CCT CCC TCA CAG GTC GAA ATT GAA ATT
 273 Pro Leu Ala Leu Leu Cys His Asp Ile Met Pro Pro Pro Ser Gln Val Glu Ile Glu Ile

1084 GGA GAA AAA AGA GTG ATT GCC TTT AAT GGG GCG GGT TTG TCT GTG GCT CCT GAA ATC AGC
 293 Gly Glu Lys Arg Val Ile Ala Phe Asn Gly Ala Gly Leu Ser Val Ala Pro Glu Ile Ser

1144 TTC GAG GAA ATT GCT GCT ACC CAC AAA AAT CCT GAG GAG GTT AGG GAG GCA TAC TCA AAG
 313 Phe Glu Glu Ile Ala Ala Thr His Lys Asn Pro Glu Glu Val Arg Glu Ala Tyr Ser Lys

1204 GCA CTG TTT GAT TCT GTG GCC ATG CAA TAC AAT GTG CTC AAA ACG GCT ATC TCC GGC AAA
 333 Ala Leu Phe Asp Ser Val Ala Met Gln Tyr Asn Val Leu Lys Thr Ala Ile Ser Gly Lys

1264 CAA GGA CTA GGA GCT TCA ACT GCG GAT GTC TCT TTG TCA CAA CCT TGG TAG
 353 Gln Gly Leu Gly Ala Ser Thr Ala Asp Val Ser Leu Ser Gln Pro Trp AM

1315 TCATTTGCAATCATTTTTCAACATCAATTCATTTGGTAATCAGGTTAGGACATAGTTTTGCATACCACAGGACAACACT
1394 GTCATTCAATTAGTACATGTACTGTAAGATAAAACCGAATTCTTTCTCTCCTCGACGTCTCGATGGATTCTGCTAAATT
1473 ACCAGCCTATATCCCACCCTAAGCACGTGCCATTTCAATCGACATCGAAGCACTTCGAATACTTGGTCTCTCATGGGAA
1552 TGTATAGATCTTTCATATCTCTTACACTAAAGGACTCGCAGAGGTTATTCGTACTTTATTTCTAAAGGTATATGTTCAC
1631 CCATTTTCATGTTTATATTATATGCTTCAAGAATTATTATGTTCAATTTGTATTATTAGTTGATACCTTTTGGCTTAGA
1710 TCATTCAGTCGAAGGTGCTCAAATCTCAACTTTGTTTTTGCTCTTAAGTTGTTGAAGGAACATTTTTAACAC
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responded to nucleotides 124–1314 and 396 amino acid residues (fig.2). The amino-terminal amino acid sequence determined for AT2 matched the amino acid sequence deduced from nucleotides 208–261. The amino-terminal amino acid sequences determined for the 18 kDa CNBr fragment of AT2 and AT3 also matched the amino-acid sequences deduced from nucleotides 322–390 and 322–363, respectively. These findings suggest that the open reading frame of this cDNA encoded a precursor of either AT2 or AT3, and that the mature enzyme consisted of 368 amino acid residues which were preceded by a leader peptide of 28 amino acid residues. The relative molecular masses of the precursor and mature proteins were calculated to be 43838 and 40929 Da, respectively. This value for the relative molecular mass of mature protein is close to the 40 kDa estimated for AT2 and AT3 by SDS-polyacrylamide gel electrophoresis and gel filtration chromatography [10]. The amino acid composition calculated from the deduced amino acid sequence of the mature enzyme was very similar to that determined for AT2 and AT3 (table 1). This result confirms that the cDNA encoded AT2 or AT3.

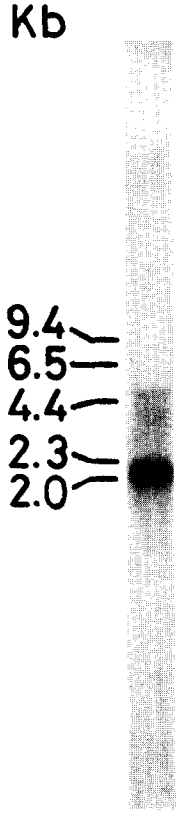


Fig.3. Blot hybridization analysis of poly(A)$^+$ RNA from squash cotyledons with a cDNA insert of λAT03.

The nucleotide sequence flanking the first ATG codon, 5′-GCAATGG-3′, is consistent with the proposed eukaryotic translation initiation sequence of (A/G)NNATGG [24]. The sequence AATAAA, implicated as a signal for polyadenylation [25], is not present in this clone. However, a sequence of ATTTTTAA (doubly underlined in fig.2), 11 bp upstream from the poly(A) tail, may be the corresponding signal sequence [26]. The nucleotide sequence in fig.2 also shows the presence of a 5′-untranslated sequence of 123 bp and a 3′-untranslated sequence of 470 bp. However, the blot hybridization analysis of squash poly(A)$^+$ RNA with a cDNA insert of λAT03 (fig.3) shows that the size of the cross-reactive mRNA was 2100 ± 50 nucleotides. This suggests that the 5′-untranslated region of the mRNA was longer than that of the λAT03 insert by 230–330 bp at maximum.

## 4. DISCUSSION

Squash glycerol-P acyltransferase has three isomeric forms, AT1–AT3. A previously study [10] indicated that AT2 and AT3 were very similar to each other in physicochemical characteristics such as relative molecular mass and isoelectric point, the values of which were about 40 kDa and p*I* 5.5–5.6, respectively. The present study shows that the amino-terminal amino acid sequence in their CNBr fragments are identical. The only difference found thus far is that AT3 but not AT2 can be adsorbed to the hydroxyapatite column. Although AT2 and AT3 are very likely to be synthesized via different genes, it is also possible that both isomeric forms are produced from the same gene but one of them is modified in some way. Since the partial amino acid sequences of AT2 and AT3 match the sequence deduced from the cDNA insert of λAT03, it is very likely that the open reading frame of the cDNA insert encodes either AT2 or AT3. Unlike AT2 and AT3, the AT1 preparation has not been purified to homogeneity [10], and therefore its amino acid sequence cannot

be determined. However, its characteristics, which have been studied with its impure preparation, appear to differ from those of AT2 and AT3. Its relative molecular mass determined by gel-filtration chromatography is about 30 kDa [10], which is much lower than that estimated for the deduced amino-acid sequence, i.e. about 41 kDa. Therefore, AT1 is unlikely to be a product of this gene.

The present study suggests that one of the isomeric forms of glycerol-P acyltransferase is synthesized as a precursor protein of 396 amino acid residues, which is processed to become the mature protein of 368 residues, losing the leader peptide of 28 amino acid residues. The leader peptides have been found in all nucleus-encoded chloroplast proteins thus far studied [27], and are postulated to be necessary for the protein to be transported from the cytoplasm to the chloroplast stroma through the envelope membrane [28]. The leader peptide of glycerol-P acyltransferase does not contain an amino acid sequence homologous to that of any other nucleus-encoded stromal protein [29]. However, the hydropathy profile of the leader peptide is similar to that of the carboxy-terminal region of leader peptides of the stromal proteins such as the ribulose-1,5-bisphosphate carboxylase small subunit [29] and spinach acyl-carrier protein I [26].

The hydrophilicity profile [30] of the deduced amino acid sequence (fig.4) does not show any cluster of hydrophobic regions which may correspond to the transmembrane structure of protein, but rather shows homogeneous distribution of hydrophilic regions. This is consistent with the finding that glycerol-P acyltransferase is a soluble protein.

Glycerol-P acyltransferase of *E. coli* is a membrane-bound protein and its DNA has been cloned [31]. The nucleotide sequence of *E. coli* glycerol-P acyltransferase contains an open reading frame of 2418 bp and predicts a polypeptide comprising 806 amino acid residues. The homology between the squash and *E. coli* glycerol-P acyltransferases in their nucleotide sequences and deduced amino acid sequences was less than 35 and 10%, respectively. Furthermore, no high homology was found even in partial sequences. A computer search of the EMBL data library [32] failed to find any nucleotide sequence homologous

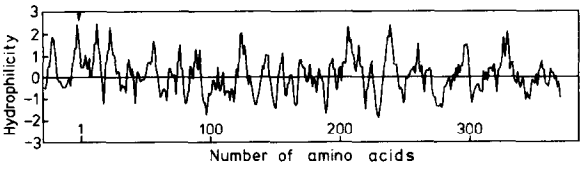


Fig.4. Hydrophilicity profile of the deduced amino acid sequence of the squash glycerol-P acyltransferase precursor. Hydrophilicity values were calculated according to the algorithm of Hopp and Woods [30] for a window size of five amino acid residues. Numbers on the abscissa denote those assigned for the deduced amino acid sequence in fig.2. The wedge denotes the predicted processing site. The overall index of hydrophilicity was +0.07.

to that of the squash glycerol-P acyltransferase cDNA.

The availability of cDNA for glycerol-P acyltransferase may open new perspectives for transformation of crop plants with respect to chilling sensitivity, since the substrate selectivity of this enzyme determines the biosynthesis of saturated and *trans*-monounsaturated molecular species of phosphatidylglycerol, which are correlated with the chilling sensitivity of plants [6].

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