Partial NH$_2$- and COOH-terminal Sequence and Cyanogen Bromide Peptide Analysis of *Escherichia coli* sn-Glycerol-3-phosphate Acyltransferase*

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Phillip R. Green†, Thomas C. Vanaman§, Paul Modrich‡, and Robert M. Bell¶

From the Departments of Biochemistry and Microbiology, Duke University Medical Center, Durham, North Carolina 27710

The committed step of phospholipid biosynthesis in *Escherichia coli* is catalyzed by the membrane bound sn-glycerol-3-phosphate acyltransferase (EC 2.3.1.15) (1, 2). A combined approach of genetics and biochemistry has been used to study the structure, function, and regulation of the glycerol-P acyltransferase. Three studies have resulted in the cloning of the glycerol-P acyltransferase structural gene (Lightner, V. A., Bell, R. M., and Modrich, P. (1983) *J. Biol. Chem.* 258, 10856–10861). Processing of the sn-glycerol-3-phosphate acyltransferase is apparently limited to the removal of the NH$_2$-terminal formylmethionine. Thirteen of 27 possible cyanogen bromide peptides predicted from the DNA sequence were purified, characterized, and assigned to their location in the primary structure. Three peptides located at positions throughout the sequence were partially sequenced by automated Edman degradation. The partial sequence analysis of the homogeneous sn-glycerol-3-phosphate acyltransferase is fully in accord with the primary structure inferred from the DNA sequence.

The NH$_2$- and COOH-terminal Sequence Determinations—To align the glycerol-P acyltransferase polypeptide within its sequenced structural gene (15), the NH$_2$-terminal sequence was analyzed by automated Edman degradation. Analysis of 26 nmol of glycerol-P acyltransferase established the sequence X-Gly-Try-Pro-X-Ile-Tyr-Tyr-Lys-Leu. An increasing background limited quantitative Edman degradation to 10 cycles. The sequence for a small amount of the protein began with methionine. The stagger detected indicates that while most of the protein is processed by cleavage of the NH$_2$-terminal methionine, at least a portion (10–20%) of the molecules arises by removal of the formyl group only. Serine and arginine were not detected. A repetitive yield of 96% was calculated from the recovery of isoleucine at cycle 6 and leucine at cycle 10 of the major sequence. If the recovery of glycine was 96% repetitive yield in cycles 6 and 7 of the major and minor sequences, respectively, an 80% first cycle yield is obtained.

The COOH-terminal residue was investigated by hydrazinolysis of glycerol-P acyltransferase. Only 0.4 mol of glycine/mol of protein was released. No amino acids were detected in the blank. Egg white lysozyme was used as a positive control.

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The abbreviations used are: glycerol-P acyltransferase, sn-glycerol-3-phosphate acyltransferase; PTH, phenylthiohydantoin; PITC, phenylisothiocyanate; DABTC, dimethylaminobenzene isothiocyanate; DABTH, dimethylaminoazobenzenethiohydantoin; HPLC, high performance liquid chromatography.

1 Portions of this paper (including "Materials and Methods," Tables I and III, and Figs. 1 and 2) are presented in miniprint at the end of this paper. Miniprint is easily read with the aid of a standard magnifying glass. Full size photocopies are available from the Journal of Biological Chemistry, 9650 Rockville Pike, Bethesda, MD 20814. Request Document No. 83M-1012, cite authors, and include a check or money order for $5.60 per set of photocopies. Full size photocopies are also included in the microfilm edition of the Journal that is available from Waverly Press.

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### Table II

| NH$_2$ terminal* | % yield | Val | Phe | Ala | Glu | Ile | Leu | Tyr | Phe |
|----------------|---------|-----|-----|-----|-----|-----|-----|-----|-----|
| NH$_2$-terminal  |         | 2   | 20  | 30  | 18  | 24  | 9   | 17  | 18  |

* HSL, homoserine lactone.
* All NH$_2$ termini found match predicted termini. The second amino acid for peptide 606-615 matches the predicted second one.
* Total nanomoles of peptide recovered as measured by amino acid analysis/330 nmol of cleaved protein.

**Fig. 3. Primary structure of glycerol-P acyltransferase.** Sequenced portions are indicated with an arrow. Underlined portions are peptides isolated and identified by amino acid analysis and partial sequencing.

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The analysis of cyanogen bromide peptides: observed and predicted amino acid compositions and NH$_2$ termini for the glycerol-P acyltransferase were determined by the double coupling Edman degradation method of Chang (20). Amino acid and NH$_2$-terminal analyses were used to identify the isolated peptides (Table II). Except for glycine which was consistently found in slightly larger amounts, the values for the amino acids agreed with those inferred from the DNA sequence. The expected NH$_2$-terminal threonine for peptide 21 was not detected, but as expected, tyrosine was detected upon a second cleavage cycle.

Three peptides from different parts of the protein were identified by amino acid analysis and partial sequencing.
subjected to quantitative automated Edman degradation. Partial sequences ranging from 12 to 21 residues in length were obtained with average repetitive yields of from 81–94% (Table III).

**DISCUSSION**

The data presented permit unambiguous alignment of the glycerol-P acyltransferase on its structural gene, plsB, and establish the primary structure as that inferred from the DNA sequence. This work comprises the first sequence analysis of any phospholipid biosynthetic enzyme. The base sequence suggested two possible start codons in the same reading frame 60 base pairs apart (15). Glycerol-P acyltransferase purified from membranes of *Escherichia coli* was subjected to automated Edman degradation (Table I). The 80% yield may indicate that up to 20% of the protein is not accessible to Edman degradation, and thus may be blocked. No evidence of PTH derivatives corresponding to the sequence starting from the first start codon was found, but protein with a sequence beginning at the second start codon was detected. While these data do not completely rule out the possibility that translation occurs from the first start codon and that the 21 NH2-terminal amino acids are post-translationally removed, the data strongly suggest that translation occurs from the second start codon.

Hydrazinolysis was used to demonstrate glycerine as the COOH-terminal residue. The predicted COOH-terminal sequence is -Thr-Gln-Gly-Glu-Gly. Although all the available evidence supports the inferred sequence and, to our knowledge, COOH-terminal processing has not been reported, it is possible that either processing or sequence assignment error could yield some other glycine as the COOH terminus. To rule out this possibility, we performed digestions with carboxypeptidases A and B on solubilized glycerol-P acyltransferase in 0.25 M potassium phosphate, pH 7.0, 0.5% Triton X-100, and 5 mM β-mercaptoethanol, on denatured glycerol-P acyltransferase in 0.1% sodium dodecyl sulfate and on glycerol-P acyltransferase bound to octyl-Sepharose CL-4B (Pharmacia) according to previously described methods (25). No amino acids were released, suggesting inaccessibility of the COOH terminus. However, the deduced sequence is further supported by the fact that 13 of 27 cyanogen bromide peptides predicted from the DNA sequence were isolated and had the correct chemical composition (Table II). The identification of three of the peptides was confirmed by partial NH2-terminal sequence analysis (Table III). These peptides would be derived from distinct sequences distributed throughout the protein accounting for 80% of the structural gene (Fig. 3). These results are in full agreement with those inferred from the DNA sequence.

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Supplementary Material to: Partial N- and COOH-Terminal Sequence and Cyanogen Bromide Peptide Analysis of Escherichia coli sn-Glycerol-3-Phosphate Acyltransferase

(P. R. Green, T. C. Vaaman, P. Modrich, and L. M. Bell)

MATERIALS

Glycerol-3-phosphate acyltransferase was purified from E. coli strain 21/2, as described [4]. Samples of enzyme were precipitated with 60% trichloroacetic acid in 1.5 ml polypropylene microfuge tubes. Triton X-100 redissolved after addition of 200 ml of 2M potassium phosphate, pH 7.4, and the precipitated enzyme was centrifuged in a Binkman Sprewendorf microfuge for 2 min. The pellet was washed five times with 1.5 ml water and dried in vacuo.

All HPLC grade solvents were purchased from Burdick and Jackson Laboratories except for phosphoric acid which was obtained from the Aldrich Chemical Co., Inc. All other chemicals were of reagent grade or better.

METHODS

N-Terminal Sequence Analysis. Automated Edman degradation was performed on 25 mg of glycerol-3-phosphate acyltransferase. The protein was dissolved in 0.1M formic acid and a portion was taken to dryness under vacuum. The residue was redissolved in 0.1M formic acid and 50 mM phosphoric acid to the reaction cup of an updated Beckman 900E sequencer incorporating improvements recommended by Shum et al. [18]. Automated degradations were performed using the 0.55 M quinhydrone buffer program. Conversion of the N-terminal amino acid derivatives to phenylthiohydantoin was carried out in 6N HCl containing 0.5% diisobutylketone at 100°C for 20 min. The precipitate was washed with acetonitrile, dried, dissolved in methanol and analyzed by HPLC on a Waters 510 HPLC liquid chromatograph (17).

Automated sequencing of peptides was performed with diethylaminoethyl buffer and using polyethylenimine [18]. CYTOSOL 5000150C] crystals containing P-arginine or P-lysine were back-hydrolyzed in phenol at 130°C for 20 h and analyzed. Fractionation of water and thiamine pyrophosphate on Amberlite IRC-50 (H+) as described [21]. The eluants were lyophilized, dissolved in 0.1N HCl and immediately analyzed by amino acid analysis.

Carboxamidomethylation and Cys Cleavage of Protein. Glycerol-3-phosphate acyltransferase (30 mg) was dissolved in 250 ml of 0.1M quinhydrone HCl, 0.1M diethylaminoethyl buffer, pH 8.2 and 1M diethylamine. The reaction mixture was allowed to stand at room temperature for 20 min. After precipitation of water and thiamine pyrophosphate, N-terminal amino acid derivatives were separated from the same reaction mixture by exhaustive dialysis against 0.1N HCl and immediately analyzed by amino acid analysis.

Isolation of Peptides. The carboxamidomethylated glycerol-3-phosphate acyltransferase was sequentially extracted with 5 ml of 0.1M MgCl2, 50-fold water excess of cyanogen bromide, 3.5 ml of 23% acetic acid, 10 ml of 6M guanidine HCl, 0.1M diethylaminoethyl buffer, pH 7.0 and 0.1M diethylamine. The extracts were combined, concentrated and dialyzed against water (pH 8.0). Peptide hydrolysate acid hydrolysate (6.5 ml) from an unopened bottle was added to the tube and evaporated. After precipitation of water and thiamine pyrophosphate, 1N hydrochloric acid was added and the reaction was carried out at 100°C for 20 min. The residue was dissolved in 1N hydrochloric acid and the reaction mixture was dialyzed against 0.1N HCl and immediately analyzed by amino acid analysis.

Carboxamidomethylated and Cys Cleavage of Protein. Glycerol-3-phosphate acyltransferase (30 mg) was dissolved in 250 ml of 0.1M quinhydrone HCl, 0.1M diethylaminoethyl buffer, pH 8.2 and 1M diethylamine. The reaction mixture was allowed to stand at room temperature for 20 min. After precipitation of water and thiamine pyrophosphate, N-terminal amino acid derivatives were separated from the same reaction mixture by exhaustive dialysis against 0.1N HCl and immediately analyzed by amino acid analysis.

Figure 1. A. Cyanogen bromide peptides prepared from 30 mg of protein were applied to a 2.5 x 90 cm column of Sephadex G-500 superfine column. The column was eluted with 0.1M MgCl2 equilibrated at 15 ml/min and monitored for OD at 280 nm. The fractions were pooled for subsequent analysis. B. The peptide from A was applied to a 2.5 x 90 cm column of Sephadex G-500 superfine column. The column was eluted with 0.1M MgCl2 equilibrated at 15 ml/min and monitored for OD at 280 nm. The fractions were pooled for subsequent analysis.
Partial Sequence of sn-Glycerol-3-phosphate Acyltransferase

![Graphs and diagrams related to the partial sequence of sn-Glycerol-3-phosphate Acyltransferase.](http://www.jbc.org/)

**Figure 1** - **A** - **E**: gel filtration profile of sn-Glycerol-3-phosphate Acyltransferase. Fractions collected from Sephadex G-75 superfine were pooled and lyophilized. After addition of water, the enzyme was dissolved in a 10% sucrose solution and applied to the column. A 0.05 M Tris buffer (pH 7.5) was used throughout. The enzyme was eluted with the buffer at 0.1 M. Fractions were collected at 10 s intervals and assayed for protein. The elution profile is shown in Figure 1A.

**Table 1**

| Cycle | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|-------|---|---|---|---|---|---|---|---|---|----|
| Actual (X| 3.7 | 3.3 | 3.7 | 3.3 | 3.7 | 3.3 | 3.7 | 3.3 | 3.7 | 3.3 |
| Residual (%) | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |

**Table 2**

| Peptide | 125-129 | 263-309 | 623-660 |
|---------|---------|---------|---------|
| Relative yield | 94% | 92% | 81% |

**Table 3**

| Peptide | 125-129 | 263-309 | 623-660 |
|---------|---------|---------|---------|
| Relative yield | 94% | 92% | 81% |

**Table 4**

| Peptide | 125-129 | 263-309 | 623-660 |
|---------|---------|---------|---------|
| Relative yield | 94% | 92% | 81% |

**Table 5**

| Peptide | 125-129 | 263-309 | 623-660 |
|---------|---------|---------|---------|
| Relative yield | 94% | 92% | 81% |

**Table 6**

| Peptide | 125-129 | 263-309 | 623-660 |
|---------|---------|---------|---------|
| Relative yield | 94% | 92% | 81% |

**Table 7**

| Peptide | 125-129 | 263-309 | 623-660 |
|---------|---------|---------|---------|
| Relative yield | 94% | 92% | 81% |

**Table 8**

| Peptide | 125-129 | 263-309 | 623-660 |
|---------|---------|---------|---------|
| Relative yield | 94% | 92% | 81% |

**Table 9**

| Peptide | 125-129 | 263-309 | 623-660 |
|---------|---------|---------|---------|
| Relative yield | 94% | 92% | 81% |

**Table 10**

| Peptide | 125-129 | 263-309 | 623-660 |
|---------|---------|---------|---------|
| Relative yield | 94% | 92% | 81% |

**Table 11**

| Peptide | 125-129 | 263-309 | 623-660 |
|---------|---------|---------|---------|
| Relative yield | 94% | 92% | 81% |
Partial NH2- and COOH-terminal sequence and cyanogen bromide peptide analysis of Escherichia coli sn-glycerol-3-phosphate acyltransferase.
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