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Structural and Functional Properties of a Phospholipase A2 Purified from an Inflammatory Exudate†

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Received September 5, 1986; Revised Manuscript Received October 8, 1986

ABSTRACT: The cell-free supernatant of sterile inflammatory peritoneal exudates contains a phospholipase A2 that participates in the digestion of *Escherichia coli* killed by polymorphonuclear leukocytes or by the purified bactericidal/permeability increasing protein (BPI) of these cells. This phospholipase A2 has been purified, and the sequence of the NH_2-terminal 39 amino acids has been determined and compared with sequences of both BPI-responsive and BPI-nonresponsive phospholipases A2 from snake venoms and mammalian pancreas. The high concentration and location of basic residues in the NH_2-terminal region is a common feature of BPI-responsive phospholipases A2 and may characterize those phospholipases A2 participating in inflammatory events.

Phospholipases such as phospholipase A2 (PLA2)[1] are thought to be involved in inflammation (Vadas & Pruzanski, 1984; Ahnfeld-Ronne & Arrigoni-Martelli, 1984; Flower, 1984). The search for means of altering phospholipase action in the host in order to modify inflammatory responses would be helped greatly by precise information about the phopsholipases involved in specific biological events. Whereas much is known about the structure and function of secretory PLA2 (Heinrikson, 1982; Dennis, 1983; Verheij et al., 1981), little is known about the phospholipases acting on the phospholipids of inflammatory cells and their targets. Most of these phospholipases are cellular enzymes that are present in trace amounts and therefore not readily available for detailed study.

Our studies on PLA, particularly PLA2, have dealt with the determinants of bacterial phospholipid degradation in relation to host defense against infection (Elsbach et al., 1979, 1985; Weiss et al., 1978, 1979; Elsbach & Weiss, 1983; Forst et al., 1982, 1986a). The antibacterial action of polymorphonuclear leukocytes (PMN) is accompanied by bacterial phospholipid degradation in which bacterial and PMN PLA participate. Using *Escherichia coli* as a test microorganism, we have found that in the PMN the activating agent for PLA's that degrade the bacterial phospholipids is a potent membrane-active and bactericidal protein that is specific for Gram-negative bacteria (Elsbach et al., 1979; Weiss et al., 1978, 1979; Elsbach & Weiss, 1983; Forst et al., 1982). The activation of PLA by this bactericidal/permeability-increasing protein (BPI) is highly selective. Of the 14 purified PLA2 that have been tested, only the PMN PLA2 and two basic snake venom enzymes can degrade the phospholipids of BPI-killed *E. coli* (Weiss et al., 1979; Forst et al., 1982; Elsbach et al., 1986). Chemical modification and primary structural analyses of the latter two enzymes have revealed a cluster of basic residues in the NH_2-terminal 15 amino acid segment that appears to be important in their action toward BPI-killed *E. coli* (Forst et al., 1986a; Elsbach et al., 1986). The scarcity of the PMN PLA2, however, has precluded similar analysis of the PLA2 of this inflammatory cell (Elsbach et al., 1979).

A much richer source of "inflammatory" PLA2 is the cell-free supernatant (ascitic fluid) of the PMN-containing sterile inflammatory exudates that can be elicited in the peritoneal cavity of rabbits (Franson et al., 1978). Added ascitic fluid can markedly enhance the degradation of phospholipids of *E. coli* killed by the PMN (Forst et al., 1986b), which suggests that its PLA2 can also contribute to bacterial phospholipid degradation. We have now purified the ascitic fluid PLA2 to homogeneity, which permits structural and functional comparison between this inflammatory exudate enzyme and BPI-responsive and -unresponsive PLA2's that have been studied previously.

Materials and Methods

Collection of Ascitic Fluid. Sterile inflammatory peritoneal exudates were collected 14–18 h after intraperitoneal injection

† This study was supported by U.S. Public Health Service Grants AI 18571 and AM 05472.
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[1] Abbreviations: BPI, bactericidal permeability increasing protein; PLA2, phospholipase A2; PMN, polymorphonuclear leukocyte; TFA, trifluoroacetic acid; HPLC, high-pressure liquid chromatography; Tris-HCl, tris(hydroxymethyl)aminomethane hydrochloride; Gdn-HCl, guanidine hydrochloride; EDTA, ethylenediaminetetraacetic acid; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N,N,N',N'*-tetraacetic acid.

0006-2960/86/0425-8381$01.50/0

Table I: Purification of Rabbit Ascitic Fluid Phospholipase A2

step	total protein (mg)	total PLA2 act. (units)	recovery (%)	sp PLA2 act. (units/mg)	purification (x-fold)
ascitic fluid	5740	1.4×10^7	100	2.4×10^3	1
CM-Sephadex	2.85	5.4×10^6	39	1.9×10^6	777
RP-HPLC	0.03[a]	4.1×10^6	31	1.4×10^8	73800

[a] Determined by amino acid analysis.

of New Zealand White rabbits with glycogen-saturated saline (Franson et al., 1978). Cell- and particulate-free supernatants (ascitic fluid) were obtained by sequential centrifugation of the exudate at 100*g* for 10 min and at 12000*g* for 30 min and stored at −20 °C.

Growth and Labeling of E. coli. *E. coli* S17, a phospholipase A⁻ mutant (Elsbach et al., 1979; Abe et al., 1974) kindly provided by Dr. S. Nojima (Faculty of Pharmaceutical Sciences, University of Tokyo), was grown in subculture in medium supplemented with either [1-^{14}C]oleic acid or [1-^{14}C]-palmitic acid to label bacterial phospholipids during growth, as previously described (Weiss et al., 1979). More than 95% of the incorporated [^{14}C]oleic acid was in the 2-ester position of bacterial phospholipids, and >90% of the incorporated [^{14}C]palmitic acid was in the 1-ester position. The labeled bacteria, resuspended in saline, were used in one of two ways: (1) as live organisms (Table III) to determine the ability of added purified PLA2 to degrade the envelope phospholipids of intact *E. coli* in the presence and absence of BPI purified from rabbit PMN (Elsbach et al., 1979) or (2) after autoclaving for 15 min at 120 °C and 2.7 kg/cm^2, which renders the envelope phospholipids readily accessible to the action of all added phospholipases A (Patriarca et al., 1972).

Assay of PLA2 Activity. PLA2 activity in ascitic fluid fractions was determined by incubation with 2.5×10^8 autoclaved [^{14}C]oleic acid labeled *E. coli* (~5 nmol of phospholipid) in a total volume of 0.5 mL, which typically contained 40 μmol of Tris-HCl buffer (pH 7.5) and 5 μmol of $CaCl_2$ (Elsbach et al., 1979). To determine the pH dependence of activity, sodium acetate–acetic acid (pH 4.0–6.0), Tris–maleate (pH 6.0–8.0), and Tris-HCl (pH 7.0–8.7) buffers were used. To determine the positional specificity of phospholipase A activity, autoclaved [^{14}C]palmitic acid and [^{14}C]oleic acid labeled *E. coli* were used as substrates. Incubations were carried out at 37 °C and terminated by the addition of 6 volumes of $CHCl_3/CH_3OH$ (1:2 v/v). Extraction, separation (by thin-layer chromatography), and liquid scintillation counting of labeled mono- and diacylphosphatides and free fatty acids were carried out as previously described (Elsbach et al., 1979). One arbitrary unit of phospholipase activity has been defined as 1% hydrolysis per hour.

Chromatographic Methods. Ion exchange chromatography of cell-free ascitic fluid was performed at 4 °C on a carboxymethyl-Sephadex C-50 column (2.5 × 14 cm) equilibrated in 0.9% NaCl that contained 2.5 mM Tris-HCl (pH 7.3) and 0.1% NaN_3. Elution of ascitic fluid protein was carried out with stepwise increasing concentrations of buffered NaCl (0.15, 0.5, and 1.5 M) and was monitored by UV absorbance (280 nm).

Reverse-phase high-pressure liquid chromatography (HPLC) of the phospholipase-rich pool obtained by ion exchange chromatography was performed on a C-4 column (Vydac; 5-μm particles, 300-Å pores) equilibrated at room temperature in 0.1% trifluoroacetic acid (TFA) and washed with a gradient of increasing acetonitrile concentration in 0.1% TFA. Details are given in the legend to Figure 1.

Electrophoretic Procedures. Electrophoresis in 13% polyacrylamide slab gels in the presence of sodium dodecyl sulfate

Table II: Amino Acid Composition of Purified Rabbit Ascitic Fluid PLA2

amino acid	mol %	amino acid	mol %
Asx	7.6	Met	0.6
Thr	5.8	Ile	1.7
Ser	6.8	Leu	5.0
Glx	5.3	Tyr	4.4
Pro	2.6	Phe	3.6
Gly	9.2	His	2.9
Ala	9.6	Lys	8.4
$^1/_2$-Cys	11.2	Arg	12.3
Val	3.0		

(NaDodSO$_4$–PAGE) was carried out according to the method of Laemmli (1970). Isoelectric focusing in polyacrylamide (6%) that contained a gradient of basic ampholines (pH 7.5–10.5) was performed as previously described (Forst et al., 1986a).

Amino Acid Analysis. Analyses were performed in a Durrum 8-500 amino acid analyzer of unoxidized and performic acid oxidized samples, hydrolyzed with 6 N HCl at 110 °C for 20 h.

Protein Determination. Protein concentration was determined either by the method of Lowry et al. (1951) with bovine serum albumin as a standard or by amino acid analysis.

Amino Acid Sequence Analysis. Purified PLA2 (1 nmol) was reduced by treatment under N_2 with 1% β-mercaptoethanol in 6 M Gdn-HCl/1 mM EDTA/0.1 M Tris (pH 6.8) at 50 °C for 1 h. After the sample was cooled to ~20 °C and flushed with N_2, [1-^{14}C]iodoacetic acid (5 μCi, 13 μmol) was added in 10 μL of 1.4 M NaOH. The sample was sealed and incubated in the dark for 30 min and then dialyzed vs. 5% acetic acid (2 L) and lyophilized. Reduced and alkylated PLA2 was applied to a filter pretreated with polybrene and subjected to Edman degradation by using an Applied Biosystems 470 A gas-phase sequencer. PTH-amino acids were identified by HPLC (Applied Biosystems 120 A PTH-analyzer). Cysteine was also identified by the radioactivity of the 1-^{14}C-labeled *S*-(carboxymethyl) derivative.

Results and Discussion

The rabbit ascitic fluid PLA2 was partially purified by ion-exchange chromatography on CM-Sephadex as previously reported (Franson et al., 1978). Large volumes of cell- and particulate-free supernatants from multiple collections of glycogen-elicited peritoneal exudates were applied to a column of CM-Sephadex equilibrated in 0.15 M NaCl that contained 2.5 mM Tris-HCl (pH 7.4). More than 99% of the total applied protein but <5% of the applied PLA2 activity were eluted in successive washes with buffered 0.15 and 0.5 M NaCl. A single peak of PLA2 activity, ca. 800-fold enriched, was recovered in ~40% yield during subsequent elution with buffered 1.5 M NaCl (Table I). This PLA2-rich eluate was further purified by reverse-phase HPLC on a C-4 column by using a linear gradient of increasing acetonitrile concentration in 0.1% trifluoroacetic acid. Approximately 80% of the applied PLA2 activity was recovered in a single protein peak (eluting at ca. 30% CH_3CN; Figure 1) with a >70 000-fold increase in specific activity (Table I). Analysis of the recovered PLA2

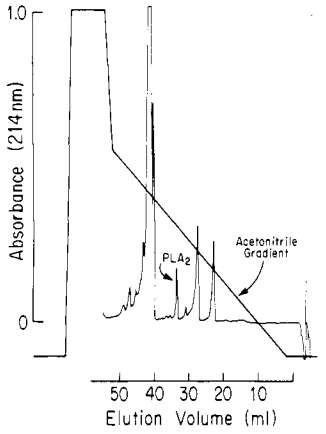

FIGURE 1: Tracing of reverse-phase HPLC (Vydac C-4 column, 5-μm particles, 300-Å pores) of PLA2-rich eluate from CM-Sephadex column which shows elution of PLA2 as a single peak. Protein elution (right → left) was monitored by absorbance at 214 nm (range 0.0–1.0). Flow rate 1 ml/min; total duration of chromatography 1 h. See text for further details.

Table III: Action of Purified Phospholipases A2 on BPI-Killed *E. coli* S17[a]

phospholipase	net phospholipid degradation (%)
ascitic fluid	9.8 ± 1.6
basic snake venom enzymes	
A. halys blomhoffii	24.2 ± 4.2
	(29 ± 5)[b]
A. halys palas	(28 ± 3)[b]
A. halys piscivorus	(0)[b]
pig pancreas	0

[a] [^{14}C]oleate-labeled phospholipase A–less *E. coli* S17 were incubated at 37 °C for 1 h with lethal amounts of BPI (1–2 μg) and ~100 arbitrary units of the indicated PLA2 (per 10^7 bacteria) in the standard incubation mixture (Elsbach et al., 1979). Net phospholipid degradation is expressed as the percent of the total recovered lipid radioactivity that accumulated as ^{14}C-labeled free fatty acid, with background levels of ^{14}C-labeled free fatty acid in untreated *E. coli* (≤4%) subtracted. The values shown represent the mean (±SEM, where indicated) of two or more experiments. [b] Data taken from manuscript submitted for publication by Forst et al.

by SDS–PAGE and isoelectric focusing revealed a single protein species with M_r 14000 and p*I* 10.5. Amino acid analysis (Table II) revealed a high content of cysteic acid (11.2%), typical of all PLA2's purified and characterized to date. The high content of arginine (12.3%) and lysine (8.4%) is consistent with the strongly basic character of this PLA2.

The positional specificity of the purified ascitic fluid PLA2 was verified, as described previously for the partially purified enzyme (Franson et al., 1978), by using [^{14}C]palmitate- and [^{14}C]oleate-labeled autoclaved *E. coli* as substrates. The labeled product of hydrolysis is almost exclusively [^{14}C]monoacylphosphatide or ^{14}C-labeled free fatty acid from [^{14}C]-palmitate- or [^{14}C]oleate-labeled autoclaved *E. coli*, respectively, consistent with the PLA2 specificity. Catalytic activity is Ca^{2+}-dependent and abolished by 2 mM EGTA. Activity is optimal between pH 7.5 and pH 8.5.

Table III shows that the ascitic fluid PLA2 can produce phospholipid degradation in a phospholipase-less mutant of *E. coli* (Abe et al., 1974), killed by purified BPI. In this respect, the ascitic fluid PLA2 resembles the rabbit PMN and basic *Agkistrodon halys blomhoffii* and *Agkistrodon halys palas* PLA2's and differs from many other purified PLA2's (including those isolated from the mammalian pancreas) that are inactive toward BPI-killed *E. coli* (Table III; Weiss et al., 1979; Forst et al., 1982; Elsbach et al, 1986).

The N-terminal 39 amino acid residues of the ascitic fluid PLA2 were determined by automated Edman degradation of the reduced and S-carboxymethylated polypeptide (Figure 2). Of 1 nmol of protein submitted to degradation, the initial yield was 70%, and the repetitive yield through the first 30 cycles was 96%. Comparison of this sequence to that in the corresponding segments of the more than 40 snake venom and mammalian pancreatic PLA2's sequenced to date reveals a high degree of homology among these enzymes from widely divergent sources and with quite different physiological roles. Conserved in all secretory PLA2's is an NH_2-terminal, amphiphilic α-helix (Dufton et al., 1983; Dykstra et al., 1978). Leu-2, Phe-5, Met-8, and Ile-9 are highly conserved residues at the lipophilic face of the helix, and these are all present in the ascitic fluid enzyme. The calcium-binding loop of PLA2 is made up in part of a glycine-rich sequence between residues 25 and 33, which defines a turn in structure (Dykstra et al., 1978). The sequence Tyr_{25}-Gly-Cys-Tyr-Cys-Gly_{30}-X-Gly-Gly_{33} is invariant in all PLA2's characterized to date with one exception, the case of the Lys-49 PLA2 (Maraganore et al., 1986; Maraganore & Heinrikson, 1986), where Tyr-28 is replaced by an Asn. The ascitic fluid PLA2 maintains this consensus sequence but, like Lys-49 PLA2, differs at position 28 where Tyr is replaced by Ser. With regard to other possible similarities between ascitic fluid and Lys-49 PLA2, it is noteworthy that the nearly invariant Gln-4 is replaced by a negative charge, an Asp and a Glu, respectively, in these enzymes.

Secretory PLA2's have been separated into two groups on the basis of differences in their patterns of disulfide bonds (Heinrikson et al., 1977; Fleer et al., 1978), a classification of enzymes that, up until the present study, was also consistent with phylogenetic separation of enzyme sources. In particular, group I PLA2's are those enzymes from mammalian pancreatic juices and venoms of elapid and hydrophid snakes that contain a disulfide bridge between half-cystines at positions 11 and 77. Group II PLA2's lack this cross-link and, instead, are characterized by a short C-terminal extension that terminates in a half-cystine cross-linked to a half-cystine at position 50; these enzymes are found in venoms of crotalid and

1				5					10					15
His	Leu	Leu	Asp	Phe	Arg	Lys	Met	Ile	Arg	Tyr	Thr	Thr	Gly	Lys
16				20					25					30
Glu	Ala	Thr	( )	Ser	Tyr	Gly	Ala	Tyr	**Gly**	**Cys**	Ser	**Cys**	**Gly**	Val
31				35				39						
Gly	Gly	(Arg)	( )	Ala	**Pro**	Lys	( )	Ala						

FIGURE 2: NH_2-terminal 39 residues of ascitic fluid phospholipase A2. Amino acid residues conserved in all sequenced PLA2's are in bold face. Unidentified or tentatively assigned residues are enclosed within parentheses.

	1				5					10					15
Ascitic Fluid	**His**	**Leu**	**Leu**	**Asp**	**Phe**	**Arg**	**Lys**	**Met**	**Ile**	**Arg**	-	**Tyr**	**Thr**	**Thr**	**Gly**	**Lys**
A.h. blomhoffii	**His**	**Leu**	**Leu**	**Gln**	**Phe**	**Arg**	**Lys**	**Met**	**Ile**	**Lys**	-	**Lys**	**Met**	**Thr**	**Gly**	**Lys**
A.p. piscivorus	**Asn**	**Leu**	**Phe**	**Gln**	**Phe**	**Glu**	**Lys**	**Leu**	**Ile**	**Lys**	-	**Lys**	**Met**	**Thr**	**Gly**	**Lys**
Bovine	**Ala**	**Leu**	**Trp**	**Gln**	**Phe**	**Asn**	**Gly**	**Met**	**Ile**	**Lys**	**Cys**	**Lys**	**Ile**	**Pro**	**Ser**	**Ser**

FIGURE 3: Comparison of NH_2-terminal 15 amino acid residues of two BPI-responsive PLA2's [ascitic fluid and *A. halys blomhoffii* (Forst et al., 1986a)] to nonresponsive PLA2's [*A. halys piscivorus* (App-D-49) and bovine pancreatic (Maraganore et al., 1986; Dykstra et al., 1978; Fleer et al., 1978)]. Amino acid residues in bold lettering are identical in two or more sequences. Basic residues are underlined. It is noteworthy that the basic PLA2 from *A. halys palas*, BPI-responsive enzyme, differs from *A. halys blomhoffii* only in the replacement of His-1 by asparagine.

viperid snakes. Since it is derived from a mammalian source, it was expected that the ascitic fluid PLA2 would have a group I disulfide pattern. However, like the group II PLA2's, the ascitic fluid enzyme lacks Cys-11, as does the PLA2 from pig ileum (Verger et al., 1982).

Proof that the ascitic fluid PLA2 is a group II enzyme was obtained by isolation and analysis of its C-terminal chymotryptic peptide, Gln-Phe-Tyr-Pro-Ala-Asn-Arg-Cys-Ser-Gly-Arg-Pro-Pro-Ser-Cys, which contains the expected (Heinrikson et al., 1977) group II extension. This finding is particularly intriguing as it demonstrates that mammalian species contain representatives of both groups of PLA2's.

The most striking finding from the partial sequence analysis carried out so far is the extensive homology in the NH_2-terminal regions of the ascitic fluid PLA2 and the two BPI-responsive snake venom PLA2's that we have previously studied (Figure 3). Of the 16 NH_2-terminal amino acids of these three enzymes, ≥70% are identical, whereas among >40 sequenced PLA2's only one residue (Ile-9) is strictly conserved (Dufton et al., 1983).

It has been suggested that the variability within this region, especially among polar residues at the surface of the PLA2, determines in large measure the differences in the interaction of PLA2 with specific biological targets (Forst et al., 1986a; Randolph & Heinrikson, 1982; van Scharrenberg et al., 1983). In the case of the basic *A. halys blomhoffii* and *A. halys palas* PLA2's, we have proposed that it is the cluster of basic amino acids (Arg-6, Lys-7, -10, -11, and -15) in this region that accounts (at least in part) for their activity toward BPI-killed *E. coli* (Forst et al., 1986a; Elsbach et al., 1986). The ascitic fluid PLA2 contains four basic residues at these sites, three of which are identical with the corresponding amino acids (Arg-6, Lys-7 and -15) in the BPI-responsive basic *Agkistrodon* enzymes. Only at position 11 is a basic residue missing (Tyr instead of Lys); this substitution perhaps accounts for the weaker activity of the ascitic fluid PLA2 toward BPI-killed *E. coli* (Table III). This remarkable degree of homology between functionally similar enzymes obtained from widely divergent sources lends further support to our concept that spatially apposed basic amino acids in the NH_2-terminal region are important determinants of PLA2 action toward BPI-killed *E. coli*. Two additional observations imply that the basic residues in this region are involved in BPI-related functions: (1) Another basic venom PLA2 (*Agkistrodon piscivorus piscivorus*), which has an amino acid sequence that is closely similar to that of the *A. halys blomhoffii* and *A. halys palas* enzymes, except for Glu instead of Arg in position 6, is inactive toward BPI-treated *E. coli* S17 (Elsbach et al., 1986); (2) Chemical modification of any one of the four NH_2-terminal lysines of the *A. halys blomhoffii* PLA2 results in extensive loss of BPI-dependent hydrolytic activity (Forst et al., 1982, 1986a) toward BPI-treated *E. coli* S17.

The ascitic fluid PLA2 resembles the PMN PLA2 that we have previously purified (Elsbach et al., 1979), not only in its activity toward BPI-killed *E. coli* but also in its apparent charge properties (basicity) (Elsbach et al., 1979) and its sensitivity to the inhibitory effect of the non-steroidal antiinflammatory drug indomethacin (Kaplan et al., 1978; Kaplan-Harris & Elsbach, 1980), to which other PLA2's are much less sensitive. PMN can secrete PLA2 (Lanni & Becker, 1983) and thus might be the source of the ascitic fluid PLA2 although other possible sources (e.g., resident peritoneal cells or serum) cannot yet be excluded. The sequence information obtained in this study should enable us to prepare specific probes for the ascitic fluid PLA2 that will help identify the origin of this enzyme and its relationship to the PMN PLA2.

Of particular interest is the finding that this PLA2, a constituent of an inflammatory exudate, which has bacteria as one of its natural targets, shares structural features with the few other PLA2's that are capable of degrading the phospholipids of BPI-killed *E. coli*. With the very recent purification from peritoneal exudates of lipocortin, an inhibitor of phospholipases, and its partial molecular characterization (Pepinsky et al., 1986), the stage has now been set for an examination of the consequences for the host of the interplay between these naturally occurring antagonists in the inflammatory process.

Registry No. Phospholipase A_2, 9001-84-7.

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Purification and Structural Properties of an Extracellular (1–4)-β-D-Mannuronan-Specific Alginate Lyase from a Marine Bacterium†

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Received May 23, 1986; Revised Manuscript Received August 25, 1986

ABSTRACT: The major extracellular alginate-degrading enzyme produced by a fermentative marine bacterium during growth on alginate as a sole carbon source has been isolated. This enzyme was purified 49-fold and was homogeneous by sodium dodecyl sulfate–polyacrylamide gel electrophoresis and high-performance liquid chromatography. The enzyme is a lyase which catalyzes depolymerization of the (1–4)-β-D-mannuronan block regions of alginate. It has an apparent native molecular mass of 29 kilodaltons, is composed of a single subunit, and has a significant fraction of potentially acidic amino acids, 14.9% Asx and 12.5% Glx. The determination of the partial amino-terminal sequence indicates the purified protein represents a single gene product. Isoelectric focusing of the purified protein yields several closely migrating forms which have p*I* values ranging from 4.2 to 5.0, suggesting posttranslational modification. The secondary structure is 74% α-helix by circular dichroism spectroscopy. The salt and pH requirements of the enzyme reflect the conditions of the marine environment in which it functions.

Alginate is a linear uronic acid polymer composed of β-D-mannuronic acid and α-L-guluronic acid in 1–4 linkages. Enzymes capable of depolymerizing alginate are almost exclusively eliminases, or lyases, and have been detected in and/or isolated from marine molluscs (Nakada & Sweeny, 1967; Muramatsu et al., 1977; Elyakova & Favorov, 1974) and from bacteria (Kashiwabara et al., 1969; Boyd & Turvey, 1977; Sutherland & Keen, 1981; Doubet & Quatrano, 1982; Hansen et al., 1984; Preston et al., 1985a; Romeo & Preston, 1986a). Previous studies utilizing these enzymes have examined the structure of alginate (Min et al., 1977; Boyd & Turvey, 1978), the composition of alginate containing cell walls of brown algae (Quatrano & Peterman, 1980), and the feasibility of generating protoplasts of brown algal species (Preston et al., 1985b; Romeo et al., 1986). The possibility that the alginate produced by *Pseudomonas aeruginosa* strains colonizing the lungs of cystic fibrosis patients is involved in the morbidity of that disease has recently led to the identification of alginate lyases in isolates of clinical origin (Linker & Evans, 1984; Dunne & Buckmire, 1985).

With few exceptions alginate lyase enzymes have been examined as impure mixtures of proteins, or even as preparations containing more than one activity, disallowing firm conclusions to be drawn about their substrate specificities, mechanisms, and structural properties. The result is that the only investigations on the structures of these enzymes, with the exception

† These studies were supported by the Gas Research Institute and the Institute of Food and Agricultural Sciences, University of Florida, as CRIS Project MCB-02183-BI and represent Journal No. 7303 of the University of Florida Food and Agricultural Sciences Experiment Station. The material is part of the dissertation submitted by T.R. to the Department of Microbiology and Cell Science, University of Florida, in partial fulfillment of the degree of Doctor of Philosophy.
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0006-2960/86/0425-8385$01.50/0