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Complete Primary Structure of Prostatropin, a Prostate Epithelial Cell Growth Factor†

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ABSTRACT: Bovine brain prostatropin is a potent and essential mitogen for prostate epithelial cell growth. The major form of prostatropin contains 154 amino acid residues in a single amino terminally blocked chain corresponding to a molecular weight of 17 400. The amino acid sequence of the 150 carboxy-terminal residues of prostatropin was derived by Edman degradation of overlapping peptides primarily generated by cleavage at lysyl and glutamyl residues. Analysis of the amino-terminal tetradecapeptide by fast atom bombardment mass spectrometry identified the blocking group as an acetyl moiety, and tandem mass spectrometry provided the sequence of the first 12 residues. Prostatropin residues 15–154 contain the sequence of bovine brain polypeptides recently described as acidic fibroblast growth factor and class I heparin-binding growth factor. The sequence of the first 25 residues of prostatropin is acetyl-Ala-(Gly, Glu)-Glu-Thr-Thr-Thr-Phe-Thr-Ala-Leu-Thr-Glu-Lys-Phe-Asn-Leu-Pro-Leu-Gly-Asn-Tyr-Lys-Lys-Pro. Reduced and carboxymethylated prostatropin exhibits mitogenic activity, suggesting that disulfide bonds among cysteine residues 30, 61, and 97 are not functionally essential. These results demonstrate by rigorous structural analysis that the brain-derived polypeptide previously described only as a mesenchymal and neuroectodermal cell mitogen is also an epithelial cell growth factor that may be involved in support of prostate hyperplasia and adenocarcinoma.

The androgen-independent proliferation of isolated epithelial cells from androgen-responsive rat prostate tumors and androgen-dependent normal prostate of rat and human requires polypeptides (prostatropins) that are concentrated in neural tissue[1] (McKeehan et al., 1984; Chaproniere & McKeehan, 1986). Two molecular forms of prostatropins were recently purified to homogeneity from bovine brain by ammonium sulfate fractionation, heparin–agarose chromatography, and reverse-phase high-performance liquid chromatography (RP-HPLC)[2] (Crabb et al., 1986). One form had a molecular weight of about 16 000 and an unblocked amino terminus, and the other form had a molecular weight of about 18 000 and a blocked amino terminus. The two forms were distributed among five chromatographic peaks and collectively consisted of about 70% blocked molecular weight 18 000 forms and about 30% unblocked molecular weight 16 000 forms. Preliminary characterization suggested that the smaller form was derived from the larger form, perhaps through proteolytic processing. Both molecular species contained regions of sequence identical with neural tissue derived, heparin-binding growth factors that have been isolated on the basis of mitogenic activity for fibroblasts and endothelial cells. Here we report the complete primary structure of the amino terminally acetylated, predominant form of bovine brain prostatropin and

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[1] W. L. McKeehan, S. Adams, and D. Fast, unpublished results.
[2] Abbreviations: aFGF, acidic fibroblast growth factor; bFGF, basic fibroblast growth factor; ECGF, endothelial cell growth factor; EDTA, ethylenediaminetetraacetic acid; FAB-MS, fast atom bombardment mass spectrometry; Gdn-HCl, guanidine hydrochloride; HBGF, heparin-binding growth factor; PEC, (pyridylethyl)cysteine; RP-HPLC, reverse-phase high-performance liquid chromatography; TPCK, *N*-tosyl-L-phenylalanine chloromethyl ketone; Tris, tris(hydroxymethyl)aminomethane.

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demonstrate that it contains within it the amino acid sequence of acidic heparin-binding growth factors recently reported by others (Gimenez-Gallego et al., 1985; Esch et al., 1985b; Strydom et al., 1986).

Experimental Procedures

Preparation of Bovine Brain Prostatropin. The amino terminally blocked form of prostatropin was isolated from bovine brain as previously described (Crabb et al., 1986). Mitogenic activity was monitored by stimulation of growth of rat normal and tumor prostate epithelial cells (McKeehan et al., 1984).

Prostatropin was reduced and cysteine residues were either pyridylethylated (Crabb & Saari, 1981), carboxymethylated, or carboxamidomethylated (Crestfield et al., 1963). For alkylation with iodoacetic acid or iodoacetamide, the dry protein was reduced in 6 M Gdn·HCl (Heico), 1 M Tris, and 0.01 M EDTA, pH 8.6, containing a 100-fold molar excess of dithiothreitol at 45 °C for 2 h, and then a 10% excess of the alkylating agent was added over total sulfhydryl content, followed shortly by another 10% excess of alkylating agent. For partial alkylations, the reaction was carried out in the absence of 6 M Gdn·HCl. The reactions were stopped by adding 2-mercaptoethanol, and the protein was desalted by RP-HPLC. Lysine residues were acylated with succinic anhydride (Crabb & Saari, 1986) for arginine-specific cleavage with trypsin.

Selective Fragmentation and Peptide Purification. For cleavage at lysine, pyridylethylated or carboxamidomethylated prostatropin was digested with 2% (w/w) endoproteinase Lys-C (Boehringer-Mannheim) in 0.1 M *N*-ethylmorpholine acetate, pH 8.6, at 37 °C for 15 h. For cleavage at glutamic acid, the succinylated and pyridylethylated growth factor was digested with 3% (w/w) *Staphylococcus aureus* V8 protease (Miles Scientific) in 1% ammonium bicarbonate, pH 8, at 37 °C for 7.5 h. For cleavage at arginine, the succinylated and pyridylethylated protein was digested with TPCK–trypsin (Cooper Biomedical) under the same conditions employed for lysine cleavage. The amino-terminal arginine peptide was subfragmented with 1% (w/w) subtilisin (Boehringer-Mannheim) in 1% ammonium bicarbonate, pH 8, and 0.1% EDTA at 37 °C for 1 h. Peptides resulting from proteolytic digests were purified by RP-HPLC on Vydac columns (The Separations Group) in aqueous trifluoroacetic acid/acetonitrile solvents (Tarr & Crabb, 1983).

Amino Acid Analysis and Edman Degradation. Phenylthiocarbamyl amino acid analysis was performed according to Tarr (1986) with a Waters Picotag system (Bidlingmeyer et al., 1984). Microsequence analyses were performed with an Applied Biosystems gas-phase sequencer and an on-line phenylthiohydantoin amino acid analyzer with the Applied Biosystems O3RPTH sequencer program and Model 120A program and solvents. Sequence analyses were carried out on 70–250 pmol of sample, and repetitive yields of 90–97% were obtained.

Mass Spectrometry. Conventional fast atom bombardment mass spectra (magnet scan on a double focusing instrument) were obtained with a VG ZAB 1F-HF mass spectrometer equipped with a standard FAB ion source and Ion Tech fast atom gun (Barber et al., 1981, 1982; Carr & Biemann, 1984). A VG 11-250 data system was used to acquire and process all data. About 1.5 nmol of the blocked amino-terminal lysyl tetradecapeptide (K1) was dissolved in 1:1 CH_3CN/H_2O, and one-third of the resulting solution was dispersed on the stainless steel target in a matrix of 3-mercapto-1,2-propanediol (Sigma Chemical Co.). The accelerating voltage of the mass spectrometer was maintained at 8 kV while 8-keV xenon atoms at a discharge current of 1 mA were used to bombard the sample. A resolution of 2000 was employed.

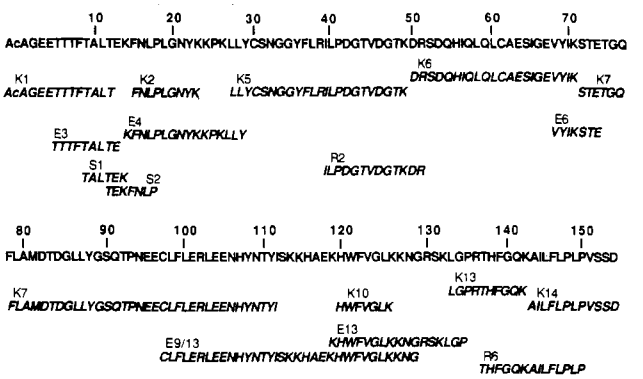

FIGURE 1: Summary of the proof of the sequence of the major form of bovine brain prostatropin. The determined sequences of specific peptides (in italics) are given in one-letter code below the summary sequence (bold type). Prefixes K, E, and R denote peptides generated by cleavage at lysyl, glutamyl, and arginyl residues, respectively; prefix S denotes subpeptides generated by subtilisin cleavage of peptide R1. The K, E, and R peptides are numbered sequentially from the amino terminus except where an uncleaved residue gives an overlap (e.g., E9/13). All peptide sequences were proven by Edman degradation except K1 where tandem mass spectrometry was used. Ac denotes an acetyl group that was identified by FAB-MS. The order of residues 2 and 3 (i.e., Gly and Glu) is not certain.

Tandem mass spectrometry was performed with a VG ZAB SE-4F, four-sector instrument of B_1E_1–C–E_2B_2 configuration, where B and E signify magnetic and electric sectors, respectively, and C represents the collision region (Boyd et al., 1986). Sample preparation and ionization were as described above, except the accelerating voltage was set at 10 kV. The pressure of He in the collision region between the two mass spectrometers was adjusted to attenuate the parent beam selected using B_1E_1 by 50%. Daughter ion spectra were obtained by a E_2/B_2-linked scan. The mass scale of the second mass spectrometer was calibrated by using the collisionally induced daughter ion spectra of cesium iodide clusters $[Cs(CsI)_n^+]$ (Boyd et al., 1986). All data were acquired with the VG 11-250 data system operating in raw data accumulation mode with a scan rate of 30 s/decade. Under these conditions between three and six scans could be accumulated per sample load. Two loadings of 250 pmol each were used to obtain the data shown in Figure 3. The resolution for this experiment was adjusted to approximately 600. The mass values determined by this procedure represent the average chemical masses for the fragments rather than the monoisotopic masses. A second set of data was accumulated with the remaining 500 pmol of peptide at a resolution of 1500 to check for the presence of previously unresolved doublets. Mass assignments were performed by computer and checked manually by inspection of oscillograph traces acquired simultaneously.

Results

The complete amino acid sequence of prostatropin from bovine brain is shown in Figure 1. The single amino terminally acetylated polypeptide chain contains 154 amino acids corresponding to a molecular weight of 17 400, in close agreement with the 17 500 ± 600 determined by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (Crabb et al., 1986). The prostatropin sequence is consistent with the amino acid composition determined experimentally (Table I).

Strategy of Sequence Analysis. The sequence of prostatropin was determined largely by Edman degradation of two primary sets of overlapping peptides generated by cleavage

Table I: Amino Acid Compositions[a] of Selected Peptides Used in the Proof of the Structure of Prostatropin

Peptide	R1	R2	E5	K6	K7	K10	K13	K14	E3	R4	R6	E13	E1	R2	R6	S1[b]	S2	prostatropin
Residues No.	1-14	15-23	27-49	50-71	72-114	120-126	133-141	143-154	5-13	14-63	68-74	119-154	1-38	39-51	137-154	9-15	12-19	
Asp/Asn (D/N)		2.0(2)	3.2(3)	1.8(2)	4.1(5)			1.8(1)		3.9(7)		c(2)	2.4(3)	2.0(3)	1.0(1)		1.5(1)	12.2(14)
Glu/Gln (E/Q)	2.7(3)			4.2(5)	6.2(8)		1.2(1)		1.0(1)	3.0(4)	1.0(1)	c(1)	3.2(3)	0.9	1.1(1)	1.3(1)	0.6(1)	17.9(18)
Ser (S)		0.6	1.0(1)	2.1(2)	3.4(3)	0.4	0.5	3.1(2)		2.0(2)	0.8(1)	4.2(3)	1.3(1)	0.7	2.2(2)	1.0		8.6(9)
Gly (G)	1.2(1)	1.5(1)	3.2(4)	2.2(1)	3.2(3)	1.2(1)	2.3(2)	0.8		5.0(5)		c(4)	4.4(4)	2.8(2)	1.4(1)	1.7	0.4	15.1(14)
His (H)				0.9(1)	1.0(1)	0.8(1)	0.9(1)			1.0(1)		1.5(2)			0.9(1)			4.8(5)
Arg (R)			1.0(1)	1.2(1)	1.6(1)		0.8(1)			1.6(2)		2.6(2)	1.3(1)	1.1(1)				5.9(5)
Thr (T)	3.7(5)		1.4(2)	0.5	2.6(5)		1.0(1)		3.4(5)	1.5(2)	0.6(1)	0.6(1)	3.6(5)	1.3(2)	0.9(1)	3.1(2)	0.5(1)	11.9(13)
Ala (A)	2.1(2)			1.1(1)	1.5(1)			1.0(1)	1.0(1)	1.1(1)		1.4(1)	2.1(2)	0.4	1.1(1)	1.0(1)		6.1(6)
Pro (P)		1.1(1)	1.0(1)		1.2(1)		1.0(1)	c(2)		2.4(3)		2.8(3)	2.2(2)	0.9(1)	2.1(2)		0.9(1)	6.7(7)
Tyr (Y)		0.9(1)	1.6(2)	1.0(1)	2.1(3)		0.5			1.9(3)	0.7(1)		2.7(3)					6.2(7)
Val (V)			1.0(1)	1.4(1)		1.0(1)		0.9(1)		1.2(1)	0.9(1)	c(2)		0.8(1)	1.0(1)			4.5(4)
Met (M)					1.2(1)													1.5(1)
Ile (I)			0.7(1)	1.9(3)	1.0(1)		0.5	0.9(1)		2.6(2)	1.0(1)	1.7(1)	1.4	0.7(1)	0.8(1)			5.7(6)
Leu (L)	1.2(1)	1.9(2)	3.1(4)	1.8(2)	4.0(6)	1.0(1)	1.0(1)	3.4(3)	1.0(1)	6.0(8)		5.3(5)	6.2(6)	1.0(1)	2.7(3)	1.1(1)	2.1(2)	18.5(20)
Phe (F)	1.1(1)	1.0(1)	0.8(1)		1.4(2)	0.8(1)	0.9(1)	1.1(1)	1.0(1)	2.0(2)		2.7(3)	3.2(3)		1.9(2)	2.2(1)	1.1(1)	7.2(8)
Lys (K)	0.8(1)	0.8(1)	0.7(1)	0.7(1)	0.9(1)	0.7(1)	0.8(1)			3.1(5)	0.7(1)	3.6(5)	2.6(4)	0.7(1)	0.7(1)	0.8(1)	0.5(1)	10.5(13)
Cys[c] (C)				d(1)	d(1)					e(2)			e(1)					3.2f(3)
Trp[c] (W)						(1)						(1)						(1)
Total residues	14	9	23	22	43	7	10	12	9	50	7	36	38	13	18	7	8	154
Amount analyzed (pmol)	54	54	58	24	14	47	70	40	82	71	58	21	65	125	74	14	29	19
Peptide recovered (pmol)	1600	336	364	248	86	295	434	404	618	531	437	208	961	936	1110	107	215	

[a] Residues per peptide by phenylthiocarbamyl amino acid analysis (6 N HCl vapor, 150 °C, 1 h). Values are from a single analysis and are uncorrected for partial destruction, incomplete hydrolysis, or contaminations from solvents (e.g., Ser and Gly). Values less than 0.4 are not reported. Numbers in parentheses are from the sequence shown in Figure 1. [b] Uncorrected for contamination with the dipeptide Thr-Phe (residues 7 to 8). [c] Not quantified due to interfering compounds. [d] Detected as carboxymethyl-Cys but not quantified due to insufficient amount. [e] Pyridylethyl-Cys (PEC) derivative coeluted with Ile. [f] Quantified as PEC by using a pH 5.6 solvent and gradient that separated PEC and Ile. [g] Not determined.

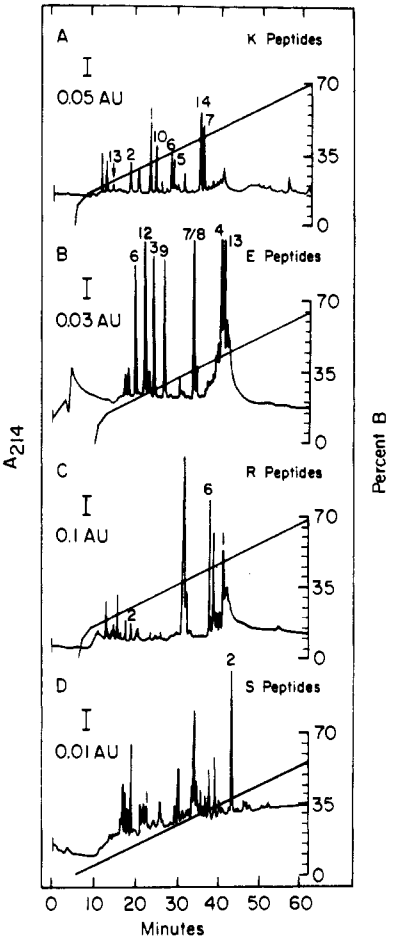

FIGURE 2: RP-HPLC fractionations of proteolytic digests of prostatropin. Separations were performed on 5-μm Vydac reverse-phase columns (4.6 × 250 mm) at room temperature and at a flow rate of 1 mL/min with the indicated gradient. Solvent A was 0.1% trifluoroacetic acid in water, and solvent B was 84% acetonitrile containing about 0.07% trifluoroacetic acid. Fractionations are (A) an endoproteinase Lys-C digest of pyridylethylated prostatropin (3 nmol) on a C_4 column, (B) a *S. aureus* V8 protease digest of succinylated and pyridylethylated prostatropin (1.6 nmol) on a C_4 column, (C) a tryptic digest of succinylated and pyridylethylated prostatropin (5.8 nmol) on a C_{18} column, and (D) a subtilisin digest of arginyl peptide R1 (900 pmol) on a C_{18} column.

at lysyl residues and glutamyl residues. Two arginyl peptides and a subtilisin subfragment were utilized for establishing further overlaps among the primary peptides. The N^{α}-acetyl blocking group and the sequence of the first four residues were determined by mass spectral analysis of a 14-residue lysyl peptide.

Lysyl Peptides. The sequence of 87 residues including two cysteines was determined by Edman degradation of peptides that were generated by treatment of carboxamidomethylated prostatropin (1 nmol) with endoproteinase Lys-C. Sequence analysis of peptides from a second endoproteinase Lys-C digest (Figure 2A) of pyridylethylated prostatropin (3 nmol) extended the identified sequence to 122 residues, which included all three cysteines (Figure 1). Lysyl peptides are numbered K1, K2, etc. from the amino terminus; yields and amino acid compositions are shown in Table I. Of the lysyl peptides, only K14 lacked a carboxy-terminal lysine, which suggested that it was the carboxy-terminal peptide. The blocked amino-terminal lysyl peptide (K1) was also purified from an endoproteinase Lys-C digest of the unmodified growth factor (6 nmol) and used for mass spectral analyses.

Glutamyl Peptides. Succinylated and pyridylethylated prostatropin (1.6 nmol) was digested with *S. aureus* V8 pro-

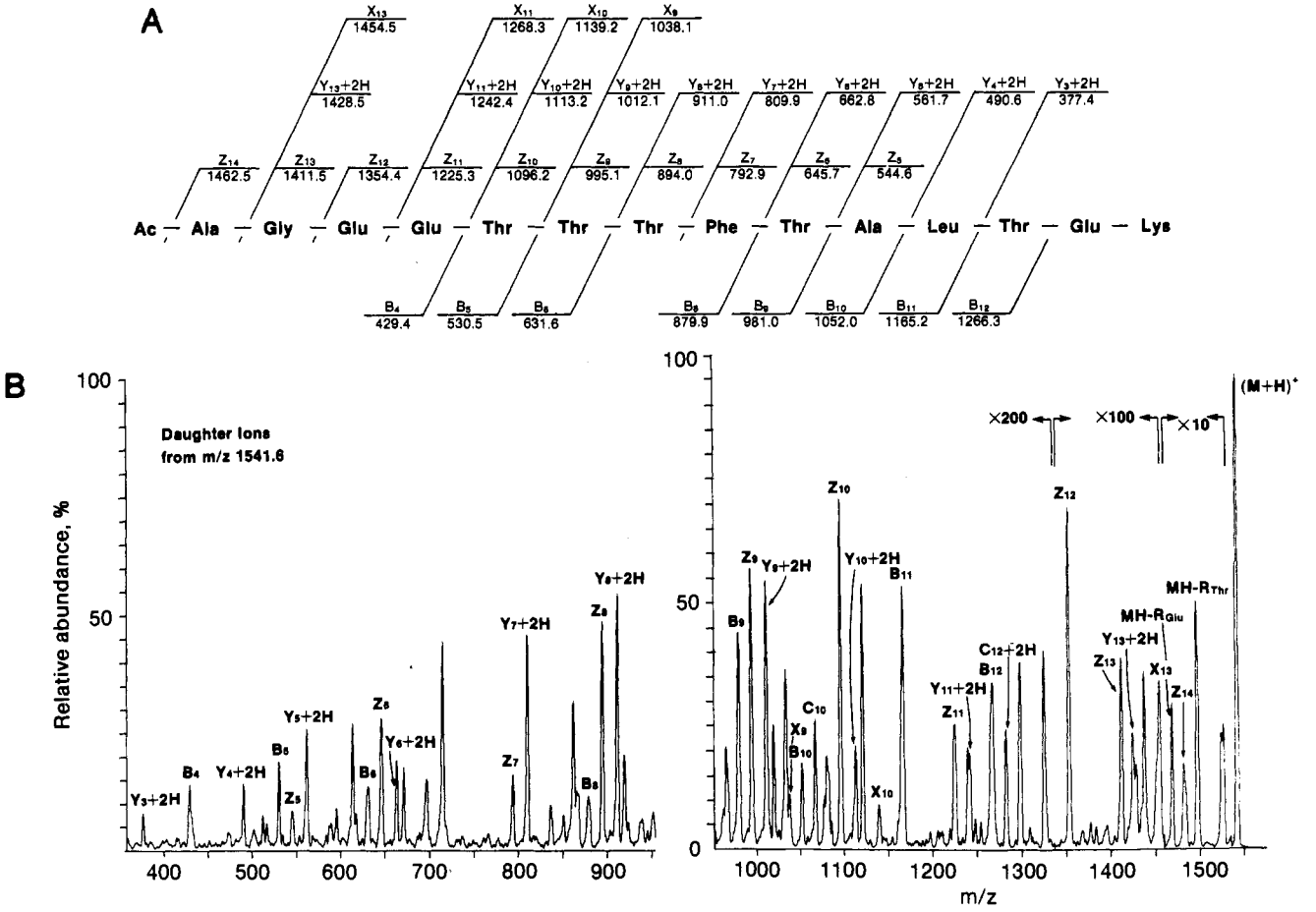

FIGURE 3: (A) Fragment ions observed in the daughter ion mass spectrum of the amino-terminal acetyl-blocked tetradecapeptide (K1). The mass values shown correspond to the calculated average chemical masses for the fragments (see Experimental Procedures). All obtained values were within ±0.4 daltons of the calculated values. (B) Daughter ion mass spectrum of the protonated molecular cluster of the acetyl tetradecapeptide (m/z 1541.6). Fragment ion nomenclature is according to Roepstorff and Fohlman (1984); B_n and C_n ions correspond to cleavage of the ...CHR_nCO–NH... and ...NH–CHR_{n+1}... bonds, respectively, with charge retained on the amino-terminal fragments. Similarly, X_n, Y_n, and Z_n correspond to fragments arising by cleavage of the ...CHR_{n+1}–CO..., ...CO–NHCHR_n, and ...NH–CHR_n... bonds, respectively, with charge retained (with or without H transfers as indicated) on the carboxy-terminal fragment.

tease, and peptides were purified by RP-HPLC (Figure 2B). Glutamyl peptides were numbered consecutively from the amino terminus (E1, E2, etc.) except for one peptide, which contained uncleaved glutamyl peptide bonds (E9/13). Glutamyl peptide E9/13 was purified from an initial V8 protease digest of pyridylethylated, unacylated prostatropin (2 nmol). Succinylation increased the extent of glutamyl cleavage in the subsequent digest (Figure 2B), apparently by enhancing the solubility of the pyridylethylated growth factor. Sequence analysis of the glutamyl peptides extended the number of identified residues to 150 of the 154 residues in the protein. Three additional overlaps were required to completely align the glutamyl and lysyl peptides.

Overlaps between Primary Peptides. To obtain the additional overlaps, succinylated and pyridylethylated prostatropin (5.8 nmol) was cleaved with trypsin at arginine residues (Figure 2C). Arginyl fragment R2 was used to link lysyl peptides K5 and K6, and arginyl peptide R5 was used to link lysyl peptides K13 and K14 (Figure 1). The final overlap was obtained by subdigesting the blocked amino-terminal arginyl peptide R1 (900 pmol) with subtilisin. Subtilisin peptides accounting for residues 7–38 of R1 were isolated (Figure 2D) and characterized. Subtilisin subfragments S1 and S2 establish the overlap between glutamyl peptides E3 and E4 and between blocked amino-terminal peptides K1 and K2 (Figure 1). On the basis of compositional analysis (Table I), the carboxy-terminal residues of S1 and S2 (i.e., Phe and Leu, respectively) were not identified by sequence analysis.

Identification of the Amino-Terminal Structure. FAB-MS revealed the chemical nature of the N^{α}-blocking group and corroborated the composition of lysyl peptide K1. The FAB mass spectrum of 500 pmol of blocked peptide K1 exhibited an intense $(M + H)^+$ ion at m/z 1540.5 ± 0.3 daltons. No sequence informative fragments were observed. The arithmetic difference between the observed molecular weight of 1539.5 and the value 1497.4 calculated from the composition indicated by amino acid analysis of K1 (Table I) and Edman degradation of E3 (Figure 1) established the molecule to be an acetyl-blocked tetradecapeptide with a composition of $Ala_2Glu_3GlyLeuLysPheThr_5$.

The sequence of the first 12 residues of the acetylated tetradecapeptide was determined by tandem mass spectrometry with collision-induced decomposition (Amster et al., 1983; Boyd et al., 1986). The first of two coupled, double-focusing mass spectrometers was used to select the desired parent ion produced by FAB ionization of 500 pmol of lysyl peptide K1. This selected parent was induced to fragment by interaction with He in the field-free region between the two mass spectrometers, and the resulting daughter fragments were transmitted into the second double-focusing combination where the ions were detected and the mass was measured. The daughter ion spectrum (Figure 3) exhibits a complete series of sequence fragments originating from the carboxy terminus beginning at residue 12 and extending to the acetylated amino terminus.

In addition, a series of acylium ion fragments from the amino terminus (B_{4-6} and B_{8-12}) are also present. These fragments (Figure 3) strongly support the sequence Ac-Ala-Gly-Glu-Glu-Thr-Thr-Thr-Phe-Thr-Ala-Leu-Thr as the first 12 amino acids of K1 and establish that the acetyl group is amide-linked to the amino terminus. Ten of the eleven other possible arrangements of the four amino-terminal residues were ruled out by the absence of one or more of the expected fragment ions (calculated with the aid of a computer program) in the daughter ion spectrum. Although not strongly supported by the data, the partial sequence Ac-Ala-Glu-Gly-Glu- cannot be completely ruled out. The fragment ions at m/z 1282.7 (presently assigned as C_{12} + 2H) and 1325.6 (no proposed structure) could correspond to the Z_{12} and X_{12} fragments, respectively, of this sequence. Significant peaks at m/z 614.0, 671.0, 696.6, 715.2, 861.8, 919.0, 1020.2, 1033.3, 1067.0, 1120.8, and 1298.4 cannot at present be assigned to any of the 12 possible sequences. These fragments apparently arise by processes other than simple cleavage of the peptide backbone and are currently under investigation.

Disulfide Bridges. The possibility of disulfide bonds among cysteine residues 30, 61, and 97 was evaluated by two general approaches. In the first approach, the unreduced and unmodified protein was digested with endoproteinase Lys-C, and some of the peptides were isolated and characterized. Two of the purified lysyl peptides, each a single peak by RP-HPLC, exhibited amino acid compositions and double sequences (i.e., K5 plus K7 and K5 plus K6), consistent with the possibility of disulfide bridges between both Cys-30/Cys-97 and Cys-30/Cys-61. Prostatropin exhibited full mitogenic activity when assayed after exposure to the alkaline digestion conditions (see Experimental Procedures) without protease. The second approach was to reduce and S-carboxymethylate or S-carboxamidomethylate prostatropin and then to assay for cell growth stimulation. Prostatropin preparations exhibited 100% activity after only reduction with dithiothreitol at pH 8.0 and repurification by RP-HPLC. Prostatropin that contained 1 or 2 mol of alkylated cysteine per mole of protein (as measured by amino acid analysis) exhibited activity equal to that of the unmodified protein, e.g., half-maximal stimulation of prostate tumor epithelial cell number at 125–300 pg of protein/mL. Quantitatively S-carboxymethylated protein (3 mol of modified cysteine per mole of protein) exhibited reduced, but still significant, mitogenic activity.

Discussion

Proof of the complete structure of the predominant molecular species of bovine brain prostatropin (prostate epithelial cell growth factor) was derived primarily from two sets of overlapping peptides generated by cleavage at lysyl and glutamyl residues. Three arginyl peptides, one of which was subdigested with subtilisin, provided the final overlaps. Fast atom bombardment and tandem mass spectrometry provided the data necessary to assign the first four amino acid residues and to determine that the protein is N^{α}-acetyl blocked. This information would have been difficult to obtain by other techniques, particularly at the picomole level. These results highlight the significant advantages of mass spectrometry when used in conjunction with conventional approaches in the microsequence analysis of posttranslationally modified proteins (Carr & Biemann, 1984). Seventy-three percent of the 154 amino acid residues in the protein were identified in more than one peptide. The weakest parts of the structural determination were the uncertainty in the order of residues 2 and 3, the lack of a direct identification of the carboxy-terminal amino acid, and the variable phenylthiocarbamyl amino acid analyses.

While this study was in progress, the sequences of various molecular forms of two classes of heparin-binding growth factor activities for fibroblasts and endothelial cells were described (Esch et al., 1985a,b; Gimenez-Gallego et al., 1985; Strydom et al., 1986). One class, referred to both as basic fibroblast growth factor (bFGF) and as class 2 heparin-binding growth factor (HBGF-2), exhibited an isoelectric pH of 8–10 and was represented by a 146-residue protein (Esch et al., 1985a). A truncated form of this cationic mitogen missing the first 15 residues has also been detected (Gospodarowicz et al., 1985). The other class of heparin-binding growth factor activities exhibited a p*I* of 5–7. Two species, a 140-residue form and a truncated form missing the first six residues, have been reported. The sequence of this acidic mitogen [variously referred to as acidic fibroblast growth factor (aFGF), endothelial cell growth factor (ECGF), and class 1 heparin-binding growth factor (HBGF-1)] has been determined by three laboratories and shown to be 53% homologous with the basic mitogen and about 27% homologous with human interleukin 1β (Esch et al., 1985b; Gimenez-Gallego et al., 1985; Strydom et al., 1986). Our results show that prostatropin residues 15–154 are identical with the carboxy-terminal 140 residues of the acidic class of heparin-binding growth factor (aFGF/HBGF-1). Therefore, the heparin-binding growth factors aFGF/HBGF-1 are likely truncated forms of prostatropin that arose by cleavage between Lys-14 and Phe-15 and between Gly-20 and Asn-21 of the intact, acetylated polypeptide. The new amino-terminal sequence information also potentially extends the homology between the two classes of heparin-binding growth factors. Prostatropin residues 10, 11, and 13 can be identically aligned with bovine pituitary bFGF residues 2, 3, and 5 (Ala, Leu, and Glu, respectively); this alignment, however, requires a three-residue gap between K1 and the rest of the prostatropin molecule.

The truncated forms of prostatropin may reflect posttranslational proteolytic processing of the 154-residue growth factor or proteolytic modification during extraction and purification. The extraction procedure employed in our study differs from that of others by the incorporation of a physiological pH (7.0) and a mixture of proteinase inhibitors (EDTA, phenylmethanesulfonyl fluoride, leupeptin, pepstatin). Since we detect no form beginning with Phe-15, this species of the growth factor would appear to be due to proteolysis during extraction and isolation.

Prostatropin contains three cysteines (residues 30, 61, and 97), and consequently three different disulfide combinations are possible. The presence of a disulfide bond between Cys-30 and Cys-97 has been suggested as a structural component of HBGF-1 (Strydom et al., 1986). We have found that prostatropin exhibits full cell growth stimulating activity after extensive reduction with dithiothreitol as well as when it contains two (carboxymethyl)- or (carboxamidomethyl)cysteine residues. The factor also retains mitogenic activity, albeit at a lower level, when quantitatively alkylated. These results suggest that disulfide bridges are not functionally essential, although it is not clear whether a specific disulfide bridge is required for maximal mitogenic activity. The apparent multiple disulfide bond combinations we observed in prostatropin after exposure to alkaline proteolysis conditions were most likely due to disulfide scrambling (Browning et al., 1986).

These results demonstrate by detailed structural analysis that neural tissue derived polypeptides previously described only as mesenchymal and neuroectodermal mitogens are also potent prostate epithelial cell growth factors. Since prostatropins are direct-acting mitogens for prostate epithelial cells,

intervention with prostatropic polypeptides at the cell membrane receptor may be a viable, new approach to therapy of both androgen-responsive and androgen-independent prostate tumors as well as benign hyperplasia of the prostate.

ADDED IN PROOF

A partial nucleotide sequence of bovine aFGF (Abraham et al., 1986) and the complete nucleotide sequence of human ECGF (Jaye et al., 1986) were published while this paper was in press. These nucleotide sequences indicate that the amino acid sequence of prostatropin residues 2 and 3 is Glu-Gly rather than Gly-Glu as presented in Figures 1 and 3.

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