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Amino Acid Sequence of Bovine Brain Derived Class 1 Heparin-Binding Growth Factor†

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ABSTRACT: The major class 1 heparin-binding growth factor from bovine brain is a single-chain polypeptide of 140 amino acids with a molecular weight of 15 877. It has the amino acid sequence Phe[1]-Asn-Leu-Pro-Leu-Gly-Asn-Tyr-Lys-Lys-Pro-Lys-Leu-Leu-Tyr[15]-Cys-Ser-Asn-Gly-Gly-Tyr-Phe-Leu-Arg-Ile-Leu-Pro-Asp-Gly-Thr[30]-Val-Asp-Gly-Thr-Lys-Asp-Arg-Ser-Asp-Gln-His-Ile-Gln-Leu-Gln[45]-Leu-Cys-Ala-Glu-Ser-Ile-Gly-Glu-Val-Tyr-Ile-Lys-Ser-Thr-Glu[60]-Thr-Gly-Gln-Phe-Leu-Ala-Met-Asp-Thr-Asp-Gly-Leu-Leu-Tyr-Gly[75]-Ser-Gln-Thr-Pro-Asn-Glu-Glu-Cys-Leu-Phe-Leu-Glu-Arg-Leu-Glu[90]-Glu-Asn-His-Tyr-Asn-Thr-Tyr-Ile-Ser-Lys-Lys-His-Ala-Glu-Lys[105]-His-Trp-Phe-Val-Gly-Leu-Lys-Lys-Asn-Gly-Arg-Ser-Lys-Leu-Gly[120]-Pro-Arg-Thr-His-Phe-Gly-Gln-Lys-Ala-Ile-Leu-Phe-Leu-Pro-Leu[135]-Pro-Val-Ser-Ser-Asp[140]-OH. The mitogen is homologous to the class 2 heparin-binding growth factor pituitary fibroblast growth factor with about 50% of the amino acids being identical between the two mitogens.

Virtually all of the polypeptide endothelial cell growth factors that have been described in the last decade can be purified rapidly and efficiently by heparin affinity chromatography (Lobb et al., 1986a,b). These heparin-binding growth factors (HBGF's)[1] have a wide range of mitogenic and nonmitogenic functions in vitro for cells of the vascular, neural, endocrine, and immune systems (Lobb et al., 1986a) and induce neovascularization in vivo [for review see Lobb et al. (1985)], suggesting that HBGF's are of broad physiological significance.

A comparison of the amino acid compositions of a number of homogeneous HBGF's from different tissues and species has shown that they all belong to one of two classes (Lobb et al., 1986a,b). Class 1 HBGF's are found in high levels in neural tissue and include mitogens designated as acidic brain FGF (Thomas et al., 1984), ECGF (Maciag et al., 1984), RDGF (D'Amore & Klagsbrun, 1984), and HGFα (Lobb & Fett, 1984). Their amino acid compositions (Thomas et al., 1984; Lobb & Fett, 1984; Lobb et al., 1986b; Burgess et al., 1985) indicate that they are closely related if not identical molecules, a conclusion that has also been reached on the basis of receptor binding and immunological cross-reactivity (Schreiber et al., 1985). Class 2 HBGF's have a wide tissue distribution and are typified by cationic pituitary FGF (Gospodarowicz et al., 1984) and cartilage-derived growth factor (Sullivan & Klagsbrun, 1985).

This paper describes the primary structure of the major class 1 HBGF found in bovine brain and shows it to be homologous to the class 2 HBGF bovine pituitary FGF (Esch et al., 1985).

Experimental Procedures

Growth Factor Isolation. Class 1 HBGF's were purified from bovine brain (Pel Freez) by homogenization, acidification to pH 4.5, ammonium sulfate precipitation, CM-Sephadex C50 cation-exchange chromatography, and heparin affinity chromatography (Lobb & Fett, 1984). Further purification

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[1] Abbreviations: ECGF, endothelial cell growth factor; HBGF, heparin-binding growth factor; RS-HBGF, reduced and S-sulfopropylated heparin-binding growth factor; RDGF, retina-derived growth factor; FGF, fibroblast growth factor; HPLC, high-performance liquid chromatography; SDS, sodium dodecyl sulfate; PITC, phenyl isothiocyanate; PTH, phenylthiohydantoin; PAGE, polyacrylamide gel electrophoresis; TFA, trifluoroacetic acid; Cys-Sp, *S*-(sulfopropyl)cysteine; Tris, tris(hydroxymethyl)aminomethane; EDTA, ethylenediaminetetraacetic acid; TLCK, N^{α}-*p*-tosyllysine chloromethyl ketone.

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by cation-exchange HPLC was carried out as described (Lobb et al., 1986a). Two class 1 HBGF's with apparent molecular weights by SDS–PAGE of 18 000 and 16 000 are purified to homogeneity by this means. Greater than 95% of the mitogen is present as the M_r 16 000 species, which stimulates half-maximal DNA synthesis in 3T3 cells at a concentration of 250 pg/mL (Lobb et al., 1986a). This major form of class 1 HBGF, which will be referred to as HBGF-1, corresponds to the mitogen previously designated as HGFα (Lobb & Fett, 1984). HBGF-1 was desalted into volatile buffer for sequencing studies by reversed-phase HPLC on a C3 column as described (Lobb & Fett, 1984). Although HBGF-1 demonstrates some microheterogeneity upon high-resolution HPLC (supplementary material Figure 4), microheterogeneous forms of the same protein were pooled for all sequencing and mapping studies.

Amino Acid Analysis. Aliquots of proteins and peptides were taken from HPLC fractions and hydrolyzed with 6 N HCl for 18 h at 110 °C as described (Strydom et al., 1985). The dried hydrolysates were derivatized with PITC (Bidlingmeyer et al., 1984; Cohen et al., 1985) and analyzed by HPLC on Waters Picotag columns (Bidlingmeyer et al., 1984). Analyses after performic acid oxidation (Moore, 1963) were performed as above except that the HPLC gradient was changed to 5% to 50% solvent B in 10 min with a convex gradient (curve 5, Waters Associates gradient controller), which separates $CySO_3H$ and Asp derivatives with base-line resolution ($CySO_3H$, 2.22 min; Asp, 2.50 min; Glu, 2.96 min).

Tryptophan was determined by the standard PITC methodology after hydrolysis with 4 N methanesulfonic acid containing 0.2% 3-(2-aminoethyl)indole (Pierce Chemical Co.) (Liu & Chang, 1971).

Reduction and S-Sulfopropylation. HBGF-1 (13–15 nmol/mL) was reduced with 1.1% tributylphosphine (ICN Biochemicals) in 0.32 M $NaHCO_3$ in 1-propanol (50% in water) containing 6 M guanidine hydrochloride and alkylated with 1,3-propanesultone (Aldrich Chemical Co.) according to Ruegg and Rudinger (1977). Reaction mixtures were dialyzed against water and concentrated by reversed-phase HPLC or desalted by molecular sieve chromatography (Strydom et al., 1985).

Proteolytic Digestion. Native HBGF-1 or RS-HBGF-1 (3–15 nmol) was dissolved in 500 mM Tris-HCl, 10 mM EDTA, and 6 M guanidine hydrochloride, pH 7.7, and diluted with 9 volumes of 1% $NaHCO_3$ (NH_4HCO_3 with protease V-8) such that the final protein concentration was 4.5–5.0 nmol/mL. TLCK-treated chymotrypsin (2% w/w, Sigma Chemical Co.), HPLC-purified trypsin (Titani et al., 1982) (3% w/w), or *Staphylococcus aureus* protease V-8 (3% w/w, Miles) was added and the mixture incubated at 35 °C. The reaction was quenched at the indicated time by addition of TFA (1.3% final concentration), unless otherwise noted, prior to fractionation by reversed-phase HPLC.

Peptide Mapping. Proteolytic digests were fractionated on a Synchropak RP-P column (Synchrom, Inc., Linden, IN, 250 × 4.5 mm), employing linear gradients with a 0.1 M perchlorate/0.1% phosphate/acetonitrile solvent system (Meek, 1980), unless otherwise noted. Peptides were desalted or further purified with TFA/acetonitrile solvent systems (Mahoney & Hermodson, 1980) on either an Altex Ultrapore IP column (Beckman Instruments Inc., 250 × 4 mm, 5-μm particle size) or an IBM octadecylsilane column (250 × 4 mm, 5-μm particle size).

Sequencing Studies. Automated microsequencing was performed with a Beckman Model 890C sequencer updated to System 890 status. The program used was Beckman 347379, revised 7/8/84, program no. 3, using 0.1 M Quadrol buffer and employing 5 mg of polybrene as carrier. Automated conversion of thiazolinones to phenylthiohydantoins employed 25% TFA containing 0.01% ethanethiol. The PTH-amino acids were identified and quantitated according to established procedures (Strydom et al., 1985). *o*-Phthalaldehyde blockage of α-amino groups at the position of proline in a sequence was performed according to Spiess et al. (1983) and Novotny and Margolies (1983).

Cyanogen Bromide Cleavage. HBGF-1 (5 nmol) was applied to the sequencer cup together with 5 mg of polybrene and dried. One cycle of Edman degradation was performed to block lysine ϵ-amino groups, 15 μL of phenyl isocyanate (Aldrich Chemical Co.) added, and one sequencer cycle performed, starting with the addition of 0.1 M Quadrol buffer. This blocks the open amino-terminal end groups (Boosman, 1980). Cyanogen bromide (50 mg) was dissolved in 500 μL of 70% TFA and added to the sequencer cup at 25 °C. After 16 h the cup was dried and one sequencer cycle, omitting the addition of PITC, was performed to remove remaining reagents and products. Normal sequencer operation was then initiated to sequence the single cyanogen bromide cleavage fragment.

Other Techniques. Hydrazinolysis and hydroxylamine cleavage were performed as described (Strydom et al., 1985).

Results

The complete sequence of the major class 1 HBGF in bovine brain is summarized in Figure 1. The calculated molecular weight is 15 877, in good agreement with its apparent molecular weight of 16 000 by SDS–PAGE (Lobb & Fett, 1984), and its calculated amino acid composition is also in good agreement with that determined experimentally (Table I). As summarized below, the sequence was established by using fragments generated from both chemical and proteolytic cleavages as well as direct Edman degradation.

Edman Degradation. Amino-terminal sequencing studies carried out on native HBGF-1 (1 nmol) allowed assignment of 26 of the first 28 residues (Figure 1). The yields of the PTH-amino acids are given in the supplementary material (Table I). Two major sequences were observed, one starting at position 7 of the other, providing internal confirmation of the second sequence. The same amino-terminal heterogeneity was observed for acidic brain FGF (Thomas et al., 1985).

Hydroxylamine Cleavage. RS-HBGF-1 (5 nmol) was digested with hydroxylamine to cleave the Asn–Gly bond found at residue 18–19, and the products were fractionated by reversed-phase HPLC (C18) to give four peptides, HY-1, HY-2, HY-2′, and HY-3′ (Figure 2, supplementary material). HY-1 (Figure 1; supplementary material Table II), an *S*-(sulfopropyl)cysteine-containing peptide, corresponded to residues 7–18 of the amino-terminal sequence and indicated that position 16 of the protein chain is a cysteine or half-cystine residue. Peptides HY-2 and HY-2′ had amino acid compositions that differed only by Asp and Ser (supplementary material Table II). Their presence indicated cleavage of HBGF-1 at a second Asn–Gly bond. Edman degradation of peptide HY-2 was interpreted through 20 cycles (Figure 1; supplementary material Table III). A separate hydroxylamine digest on native HBGF-1 (5 nmol) was fractionated by molecular sieve and reversed-phase HPLC. HY-1 was not found, suggesting a disulfide bond to the larger fragment through Cys-16. Sequencer degradation of the high molecular weight fragment, HY-3, which included *o*-phthalaldehyde blocking at cycle 9, allowed the amino-terminal sequence to be extended to residue 48, as shown in Figure 1 (supplementary material

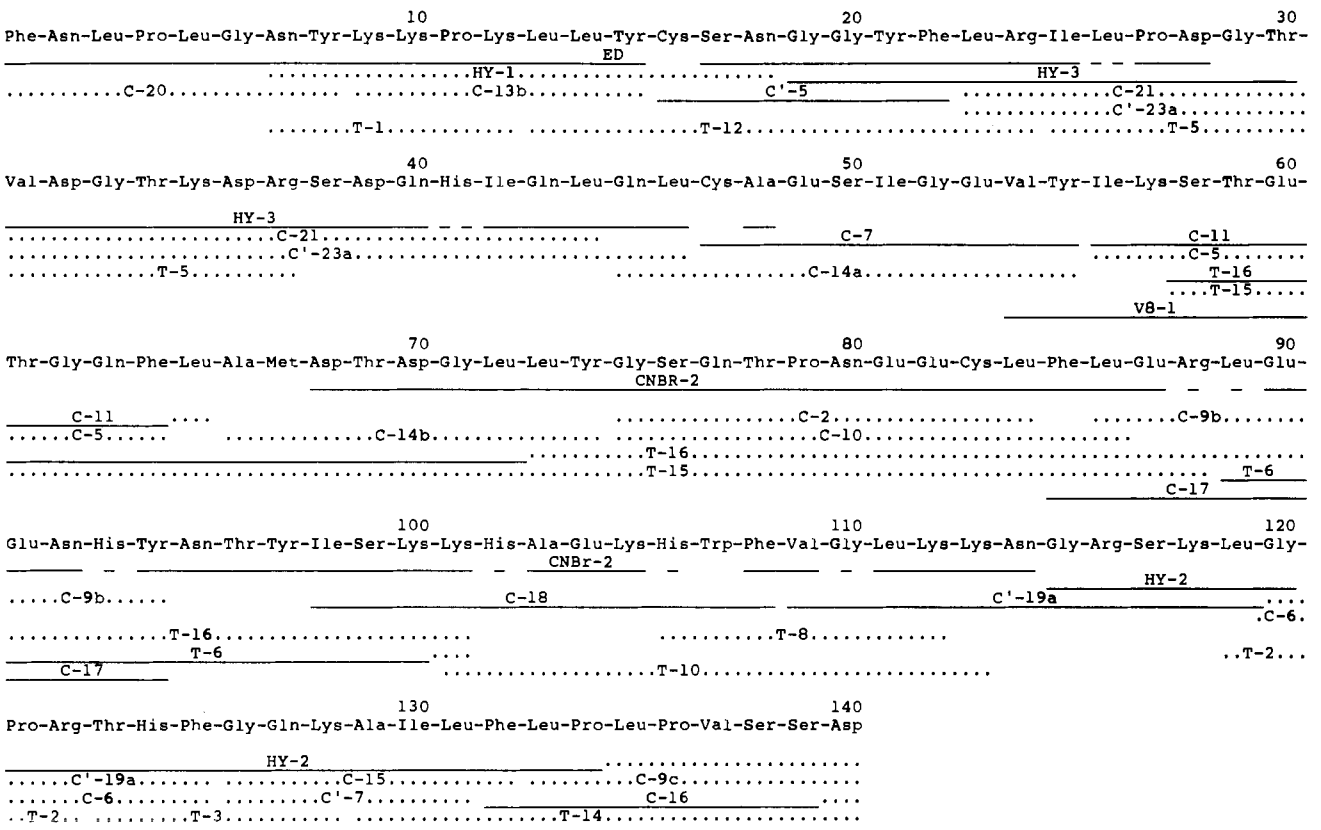

FIGURE 1: Amino acid sequence of bovine brain HBGF-1. The extent of sequencing of cyanogen bromide (CNBr), hydroxylamine (HY), chymotrypsin (C), trypsin (T), and protease V-8 (V8) digest products and of the amino terminus (ED) is indicated by solid lines. Dashed lines indicate where tentative assignments were made, and gaps indicate where no assignment could be made. The extent of Edman degradation of partially sequenced peptides is indicated by a solid line with a dotted line extending to the peptide carboxyl terminus as determined by amino acid composition. Dotted lines alone denote sequences confirmed by amino acid composition of peptides. Chymotryptic peptides from 24-h (Figure 2a) and 3-h (supplementary material Figure 2) digests are indicated by C and C′, respectively.

Table IV). Consistent with the presence of a disulfide bond, this degradation gave three sequences beginning at Gly-19, Phe-1, and Asn-7.

Cyanogen Bromide Cleavage. Cyanogen bromide cleavage at the single methionine (Table I) of HBGF-1 was performed in the sequencer cup (see Experimental Procedures). The fragment generated was sequenced through 47 cycles (Figure 1, CNBr-2; supplementary material Table V). At cycle 9 the cleavage step (Beckman program step 213) was shortened from 2 min to 0 min to avoid cyclization of Gln-10 which, in preliminary experiments, readily cyclized to pyroglutamic acid. At cycle 16 the HPLC chromatogram of the PTH derivative contained peaks associated with the sequencer-derived product of cysteine.

Proteolytic Cleavage. From amino-terminal and cyanogen bromide and hydroxylamine fragment sequencing, 112 residues had been assigned in three large nonoverlapping sections. Eight additional residues had been tentatively placed. The remaining sequences and overlaps were determined by proteolytic fragmentation.

Various chymotryptic, tryptic, and *S. aureus* protease V-8 digests of native HBGF-1 or RS-HBGF-1 were fractionated by reversed-phase HPLC. The resultant peptide maps for extensive (24-h) chymotryptic and tryptic digests are shown in parts a and b of Figure 2, respectively. The amino acid compositions of the peptides from the chymotryptic digests account for all but three amino acids of the polypeptide chain (Figure 1; Table I). Further peptide maps and peptide compositions are provided in supplementary material Figures 2 and 3 and supplementary material Table VI. Sequence studies on some of the chymotryptic, tryptic, and protease V-8

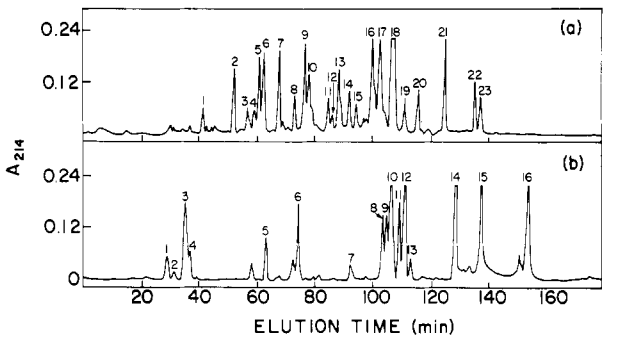

FIGURE 2: (a) Fractionation of a chymotryptic digest (24 h) of RS-HBGF-1 (15 nmol) by HPLC on a Synchropak RP-P (C18) column. Solvent A was 0.1 M perchlorate and 0.1% orthophosphoric acid (pH 2.5), and solvent B was 75% acetonitrile and 25% solvent A. The gradient was linear from 0% to 50% solvent B in 3 h at a flow rate of 0.8 mL/min. The fractions that contained significant quantities of amino acids after hydrolysis are numbered according to their order of elution. (b) Fractionation of a tryptic digest (24 h) of native HBGF-1 (13 nmol) by HPLC on a Synchropak RP-P (C18) column. At the end of the digest period, the solution was made 1 mM in dithiothreitol and allowed to stand for 30 min at 25 °C before quenching with TFA (1.3%). Solvents A and B were employed. The gradient was linear from 0% to 50% solvent B in 160 min. The fractions containing peptides are labeled.

cleavage products (Figure 1; supplementary material Table VII) provided the necessary information for completion of the HBGF-1 sequence. Thus, peptides C-7, C-11, T-16, and V8-1 provided overlaps between the amino-terminal sequence and the cyanogen bromide fragment (CNBr-2) starting at Asp-68, while C′-19a overlapped CNBr-2 with the carboxyl-terminal peptide, HY-2. The sequence studies on peptides C′-5, C-17,

Table I: Amino Acid Compositions of Chymotryptic Peptides of Bovine HBGF-1[a]

	peptide													bovine HBGF-1		
	C-20	C-13b	C′-5	C′-23a	C-7	C-11	C-14b	C-2	C-17	C-18	C′-19a	C-15[b]	C-9c	from chymotryptic peptides	from sequence[c]	amino acid analysis[d]
Asp	2.38 (2)		0.89 (1)	3.85 (4)	0.11	0.49	1.96 (2)	1.24 (1)	1.03 (1)	0.47	1.14 (1)	0.32	0.86 (1)	13	14	14.2
Glu				2.94 (3)	2.09 (2)	2.26 (2)	0.17	3.13 (3)	2.99 (3)	1.48 (1)	0.14	1.13 (1)		15	15	17.3
Ser		0.43	1.71 (1)	1.56 (1)	1.08 (1)	1.00 (1)	0.17	1.00 (1)	0.84	1.38 (1)	1.04 (1)	0.38	2.25 (2)	9	9	8.9
Gly	1.96 (1)		(2)[e]	2.69 (2)	1.30 (1)	1.64 (1)	1.20 (1)	1.13 (1)		0.76	2.99 (3)	1.28 (1)		12	13	14.0
His				0.81 (1)					0.75 (1)	1.79 (2)	0.89 (1)	0.07		5	5	5.4
Arg				1.78 (2)		0.15	0.10		0.99 (1)		1.92 (2)	0.18		5	5	5.2
Thr				2.01 (2)		1.83 (2)	1.10 (1)	1.22 (1)	0.32		0.98 (1)	0.17		7	8	8.7
Ala				0.59	1.01 (1)	0.21	0.99 (1)		0.39	0.71 (1)	0.16	1.27 (1)		4	4	4.7
Pro	1.04 (1)	1.35 (1)		2.08 (1)		0.16	0.14	0.87 (1)	0.28		1.11 (1)	0.19	2.07 (2)	7	7	7.0
Tyr	0.98 (1)	1.21 (1)	1.39 (1)	0.15	0.93 (1)	0.29	0.94 (1)		0.92 (1)		0.03	0.16		7	7	6.7
Val				1.00 (1)	0.96 (1)				0.28		0.98 (1)	0.26	0.95 (1)	4	4	4.2
Met				0.13			0.8 (1)				0.02	0.08		1	1	1.2
Ile				1.81 (2)	1.11 (1)	0.99 (1)	0.17		0.39	1.37 (1)	0.10	0.85 (1)		6	6	6.0
Leu	2.16 (2)	2.00 (2)		3.71 (4)		1.15 (1)	2.01 (2)	0.88 (1)	1.99 (2)		1.95 (2)	1.06 (1)	2.09 (2)	19	19	18.9
Phe	0.94 (1)		1.10 (1)	0.26		0.94 (1)			0.99 (1)	0.99 (1)	1.04 (1)	0.85 (1)		7	7	6.9
Trp										(1)[e]				1	1	0.8[g]
Lys		2.71 (3)		1.20 (1)		0.82 (1)			0.32	3.10 (3)	3.12 (3)	1.01 (1)		12	12	13.0
$^1/_2$-Cys[f]			1.03 (1)		0.72 (1)			0.55 (1)						3	3	2.6[h]
pmol analyzed	24	35	30	17	108	11	80	50	83	42	65	60	30			
sequence position	1–8	9–15	16–22	23–46	47–55	56–65	66–74	75–84	85–94	98–108	109–125	126–132	133–140			

[a] Relative molar amounts of amino acids are given. Peptides labeled C and C′ are from extensive (24-h) and limited (3-h) digests, respectively. Peptides are numbered according to their HPLC elution order [see Figure 2a (24-h digest) and Figure 2 in supplementary material (3-h digest)]. Unless otherwise noted, peptides were rechromatographed with volatile buffers (TFA/acetonitrile; Mahoney & Hermodson, 1980) prior to amino acid analysis. Analyses are not corrected for Gly, Ser, Ala, and Asp, which are present at this level in some of the HPLC fractions. Numbers in parentheses indicate the expected number of residues from sequence. [b] Amino acid analysis performed directly on aliquots from initial HPLC fractionation (Figure 2a). [c] See Figure 1. [d] From Lobb and Fett (1984); normalized to 140 amino acids, unless otherwise noted. [e] From sequence determination. [f] Determined as *S*-(sulfopropyl)cysteine (Cys-Sp) unless otherwise noted. [g] Tryptophan was determined by amino acid analysis as described under Experimental Procedures. [h] Determined as cysteic acid. Amino acid analysis of HPLC-purified, reduced, and S-sulfopropylated HBGF-1 gave 2.6 Cys-Sp residues.

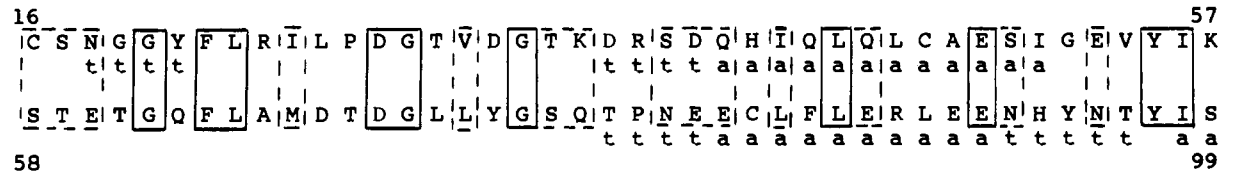

FIGURE 3: Alignment of the internally duplicated regions of bovine brain HBGF-1. Under the sequences the symbols t and a indicate predicted regions of β-turns and α-helices, respectively. Identities are boxed, while similarities are enclosed by dashed lines. Similarities are defined either as chemical similarity or according to the mutation data scoring matrix of Schwartz and Dayhoff (1978).

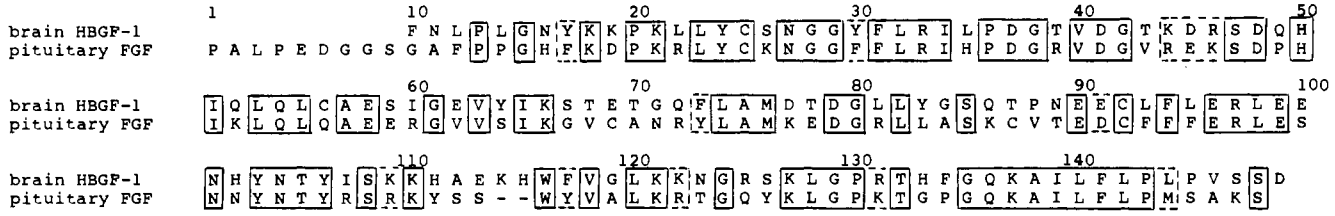

FIGURE 4: Alignment of the amino acid sequence of bovine HBGF-1 with bovine pituitary FGF (Esch et al., 1985). One gap was introduced at position 114–115 to maximize homology. The one-letter amino acid notation is used. Identities between HBGF-1 and pituitary FGF are boxed, while similarities are enclosed by dashed lines.

T-6, C-18, and C-16 confirmed sequence assignments in the chemical cleavage fragments, and amino acid compositions of, e.g., peptides T-5, C-21, C′-23a, C-14a, and T-14 likewise corroborated assignments in their portions of the polypeptide chain.

Carboxyl-Terminal Determination. Carboxyl-terminal amino acid determination by hydrazinolysis indicated aspartic acid as the carboxyl-terminal residue, although significant amounts of serine and glycine were also found. Carboxyl-terminal heterogeneity was also present in some of the HBGF-1 preparations. For example, two carboxyl-terminal hydroxylamine digest fragments were identified, HY-2 and HY-2′, which differed in composition by Asp and Ser (supplementary material Table II). In addition, two tryptic peptides lacking Arg or Lys and also differing in composition by Asp and Ser were found in one tryptic digest. Thus, one form of HBGF-1 lacks the carboxyl-terminal Ser-Asp dipeptide.

Half-Cystine Content. Amino acid analysis of HBGF-1 after reduction, S-sulfopropylation, and purification by reversed-phase HPLC indicates the presence of three half-cystines (Table I), as predicted by the sequence (Figure 1). However, Thomas et al. (1984) reported the presence of four half-cystines in acidic brain FGF, determined as cysteic acid after performic acid oxidation. Moreover, amino acid analysis of pituitary FGF indicated the presence of six half-cystines as cysteic acid, but only four after further purification by reversed-phase HPLC (Esch et al., 1985). Thus, the cysteic acid content of HBGF-1 was determined after performic acid oxidation but without further purification. Only three half-cystines were found (Table I).

Discussion

The sequence of the major class 1 heparin-binding growth factor from bovine brain was determined in three stages. First, direct Edman degradation identified 26 residues at the amino terminus. Second, hydroxylamine cleavage demonstrated the presence of two Asn–Gly bonds and, in conjunction with cyanogen bromide cleavage at the single methionine, allowed the identification of about 85% of the sequence utilizing these chemical cleavage products alone. Third, proteolytic fragmentation confirmed the known sequences, established sequences of unknown regions, and provided the final overlaps. Although the chymotryptic peptides gave all but three amino acids of the sequence, two further digests with trypsin and protease V-8 were required to provide two critical overlaps. For this purpose, the combination of peptide mapping by HPLC with rapid, highly sensitive amino acid analysis after precolumn derivation with PITC (Bidlingmeyer et al., 1984) proved particularly powerful. It allowed the rapid selection and purification of those peptides critical for the completion of the sequence.

The amino- and carboxyl-terminal sequences of HBGF-1 are identical with those recently reported for acidic brain FGF (Bohlen et al., 1985; Esch et al., 1985; Thomas et al., 1985). Moreover, the amino-terminal heterogeneity observed here with HBGF-1 was also found in one of these studies (Thomas et al., 1985). These results confirm our original suggestion (Lobb & Fett, 1984) that bovine brain acidic FGF and bovine brain HBGF-1 (which we originally termed HGFα) are identical.

Isoelectric focusing studies of both partially purified and homogeneous class 1 HBGF's have consistently indicated p*I*'s of about 5 (Thomas et al., 1984; Conn & Hatcher, 1984; Maciag et al., 1984; D'Amore & Klagbrun, 1984). However, the sequence of HBGF-1 contains 16 acidic residues (Asp, Glu) and 17 basic residues (Lys, Arg), suggesting a p*I* of about 7. The reason for this discrepancy is unclear at present.

Inspection of the sequence of HBGF-1 shows an internal duplication by aligning Ser-58 with Cys-16, creating an overlap of 42 residues (Figure 3). This alignment, which contains no deletions, is supported by 10 identities (24%) and a further 14 similarities, for a total of more than 50% similarity. This similarity is also reflected in empirical structure predictions according to Chou and Fasman (1978), which predict α-helical regions, among others, for the aligned residues 40–51 and 82–91, each preceded by a β-turn at residues 36–39 and 78–81, respectively.

The recent publication (Esch et al., 1985) of the complete sequence of bovine pituitary FGF, a class 2 HBGF (Lobb et al., 1986b), allows its detailed comparison with the sequence of a class 1 mitogen (Figure 4). The two sequences are homologous, and if a single deletion of two residues is inserted at position 114–115 of the pituitary FGF sequence, half of the amino acid residues in the two sequences are identical. We will discuss the features of these sequences with reference to the alignment numbering in Figure 4.

The largest continuous stretch of identity (residues 135–143) is found near the carboxyl termini of the two proteins, in the region from 127 to 143, where 14 out of 17 residues are identical. The largest stretch of dissimilar sequence is located

between residues 59 and 73 where only 4 of the 15 residues are identical. Two of the half-cystinyl residues are aligned between the two proteins, suggesting a conserved disulfide bond between Cys-25 and Cys-92. Consistent with this suggestion, hydroxylamine cleavage studies indicate that Cys-25 is indeed disulfide bonded (see Results), while cyanogen bromide cleavage studies (Burgess et al., 1985) show that the disulfide bond spans the methionine residue, placing the other half-cystine at Cys-92. If this is the case, Cys-56 of HBGF-1 either exists as a free sulfhydryl or is bound to exogenous cysteine or some other moiety by a disulfide, as has been suggested to occur with two half-cystinyl residues in pituitary FGF (Esch et al., 1985). However, amino acid analyses are inconsistent with the latter interpretation. Moreover, reducing agents stabilize acidic brain FGF at high pH values (8–9) against loss of mitogenic activity (Lemmon et al., 1982), while pituitary FGF shows no loss of mitogenic activity under the same conditions. These results imply not only the presence of a labile free cysteine in class 1 HBGF's but also the presence of a single disulfide bond in both class 1 and class 2 HBGF's.

The Gly-15–His-16 bond of pituitary FGF is susceptible to cleavage (Esch et al., 1985), and in the case of pituitary FGF from corpus luteum and kidney, the truncated protein is the major form isolated. Importantly, this same position in HBGF-1 (Gly-15–Asn-16; Figure 4) is also susceptible to cleavage. As isolated, microheterogeneous forms of HBGF-1 beginning with Phe-10 and Asn-16, respectively, are found in approximately equal quantities (supplementary material Table IX; Figure 4). Significantly, a second form of class 1 HBGF of higher molecular weight can also be isolated from bovine brain (Burgess et al., 1985; Lobb et al., 1986a). In our SDS–PAGE system this mitogen of M_r 18 000 has a mobility identical with that of brain-derived class 2 HBGF (Lobb et al., 1986a). It is possible that this form of HBGF-1 might have an extended amino terminus with homology to residues 1–9 of pituitary FGF. Whether this microheterogeneity is due to incomplete processing of precursors or to proteolytic cleavage during purification is not clear, but the former is more likely, since the cleavage site is the same in both classes of mitogens.

The primary structures of several other heparin-binding proteins, including bovine platelet factor 4 (Deuel et al., 1977; Hermodson et al., 1977) and human antithrombin III (Chandra et al., 1983), are known. While chemical modification studies indicate the importance of positively charged amino acids in heparin binding (Handin & Cohen, 1976; Pecon & Blackburn, 1984; Rosenberg & Damus, 1973; Jorgensen et al., 1985; Schwarzbauer et al., 1983), no short linear portion of these sequences has been unequivocally shown to be the heparin binding site. However, clusters of basic residues (Lys, Arg) in combination with aromatic and/or hydrophobic residues are likely candidates for heparin binding sites [for examples see Deuel et al. (1977) and Schwarzbauer et al. (1983)].

In considering potential heparin binding sites in HBGF-1, two regions containing clusters of basic residues are quite pronounced. The region from residue 109 to residue 131 (Figure 4) is highly basic and contains 10 lysine, arginine, or histidine residues, including 2 Lys-Lys sequences. A second basic cluster is found near the amino terminus (Lys-18–Lys-21) and is flanked by hydrophobic residues. These two clusters in HBGF-1 have counterparts in basic FGF, although the exact positions and identities of the basic residues differ in several positions. Esch et al. (1985) have suggested that these same two domains in pituitary FGF may be involved in heparin binding. It should be noted that a disulfide bond between Cys-25 and Cys-92 would bring these two basic clusters in close proximity.

Most of the acidic residues in HBGF-1 are localized between residues 41 and 100, with a highly acidic cluster between residues 90 and 100. Importantly, 10 of these acidic residues are conserved in basic FGF (Figure 4). While the importance of this acidic region remains undefined, groups of acidic and basic clusters have been seen with another heparin binding protein, platelet factor 4 (Deuel et al., 1977; Hermodson et al., 1977).

The sequence of HBGF-1 was compared with those of proteins in the National Biomedical Research Foundation Databank, employing the methodology of McLachlan (1971) and therefore specifically searching for structural similarities. Many short regions of similarity were observed, but none showed convincing homology. The strongest segment of similarity found was between residues 93–117 of HBGF-1 and residues 78–102 of ribosomal protein L21 of *Escherichia coli* (Heiland & Wittmann-Liebold, 1979). At a span width of 21 the probabilities for random matches range down to 10^{-7}. While this similarity suggests that these two regions are structurally related, the functional significance of this observation is unknown. The amino-terminal and carboxyl-terminal regions of acidic brain FGF show some similarity to human interleukin 1 (IL-1) (Thomas et al., 1985). However, by use of the method of McLachlan (1971), no convincing homology is found when the complete sequence of HBGF-1 is compared with IL-1. Finally, no homology was found between HBGF-1 and angiogenin (Strydom et al., 1985; Kurachi et al., 1985), a human tumor derived protein with potent angiogenic activity in vivo (Fett et al., 1985).

The primary structure of the major class 1 HBGF from bovine brain, in conjunction with that of the class 2 HBGF bovine pituitary FGF (Esch et al., 1985), provides a structural basis for investigating the functional similarities and differences between the two mitogens, which are typical of all class 1 and class 2 HBGF's.

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Supplementary Material Available

Tables I, III–V, VII, and IX containing yields of PTH-amino acids by Edman degradation, Tables II, VI, and VIII giving amino acid compositions of peptides, and Figures 1–4 showing chromatographic separations of peptides and proteins (16 pages). Ordering information is given on any current masthead page.

Registry No. Class 1 HBGF, 100205-31-0.

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