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Human Class 1 Heparin-Binding Growth Factor: Structure and Homology to Bovine Acidic Brain Fibroblast Growth Factor†

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ABSTRACT: The structure of the major class 1 heparin-binding growth factor from human brain has been analyzed. Edman degradation performed on the native mitogen and on fragments generated by chemical and enzymatic cleavage allows the sequence to be described by four nonoverlapping segments. The sum of the amino acids of the four segments is in excellent agreement with the experimentally determined amino acid composition of the mitogen itself, suggesting that, jointly, they account for the entire molecule. The four segments can be aligned into a presumptive complete sequence that shows 92% identity with that of bovine acidic brain fibroblast growth factor. The data indicate that the human mitogen has the following sequence: Phe[1]-Asn-Leu-Pro-Pro-Gly-Asn-Tyr-Lys-Lys-Pro-Lys-Leu-Leu-Tyr[15]-Cys-Ser-Asn-Gly-Gly-His-Phe-Leu-Arg-Ile-Leu-Pro-Asp-Gly-Thr[30]-Val-Asp-Gly-Thr-Arg-Asp-Arg-Ser-Asp-Gln-His-Ile-Gln-Leu-Gln[45]-Leu-Ser-Ala-Glu-Ser-Val-Gly-Glu-Val-Tyr-Ile-Lys-Ser-Thr-Glu[60]-Thr-Gly-Gln-Tyr-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]-Asn-Trp-Phe-Val-Gly-Leu-Lys-Lys-Asn-Gly-Ser-Cys-Lys-Arg-Gly[120]-Pro-Arg-Thr-His-Tyr-Gly-Gln-Lys-Ala-Ile-Leu-Phe-Leu-Pro-Leu[135]-Pro-Val-Ser-Ser-Asp[140].

Heparin-binding growth factors (HBGF's)[1] are mitogens for a variety of mesoderm-derived cells in vitro and can induce neovascularization in vivo [for review, see Lobb et al. (1986a)]. They are characterized by an unusual affinity for the complex glycosaminoglycan, heparin, and this affinity has been exploited for their purification and characterization from a variety of tissues and species (Lobb et al., 1986a). HBGF's can be grouped into two classes on the basis of a number of criteria (Lobb et al., 1986b). Class 1 HBGF's are found in high levels in neural tissue and are typified by bovine acidic brain FGF (Thomas et al., 1984). Class 2 HBGF's are found in a variety of tissues and are exemplified by bovine pituitary FGF (Gospodarowicz et al., 1984) and cartilage-derived growth factor (Sullivan & Klagsbrun, 1985).

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[1] Abbreviations: HBGF, heparin-binding growth factor; RS-HBGF, reduced and S-sulfopropylated heparin-binding 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-HCl, tris(hydroxymethyl)aminomethane hydrochloride; EDTA, ethylenediaminetetraacetic acid.

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The primary structures of mitogens typical of both classes, bovine acidic brain FGF (Gimenez-Gallego et al., 1985; Esch et al., 1985a; Strydom et al., 1986) and bovine pituitary FGF (Esch et al., 1985b), have recently been determined. The two sequences are distinct, but homologous, with about 50% identity of amino acids (Esch et al., 1985a; Strydom et al., 1986). Despite the intrinsic importance of structural studies on human HBGF's, only limited sequence information has been published to date and only on the class 2 HBGF from human brain (Bohlen et al., 1985). Moreover, a comparison of the primary structures of HBGF's from different species would provide further insight into the role both of the overall structure and of specific amino acids in function. Therefore, in this paper detailed structural analysis of the major human brain-derived class 1 HBGF (HBGF-1) is described.

Materials and Methods

Human Brain HBGF-1 Isolation. Human brain tissue was obtained at autopsy and stored at −20 °C. Tissue in 600–1000-g lots was homogenized, acidified to pH 4.5, and processed through ammonium sulfate precipitation and CM-Sephadex C-50 cation-exchange chromatography as described previously (Lobb et al., 1985b). The HBGF-containing pool was applied to heparin–Sepharose (Pharmacia) and the column washed with 10 mM Tris-HCl and 0.6 M NaCl, pH 7.0. Subsequently, the column was eluted at a flow rate of 10–14 cm/h with 10 mM Tris-HCl, pH 7.0, containing first 0.8 M NaCl, then 1.2 M NaCl, and finally 2.0 M NaCl (Lobb et al., 1986a). The HBGF-1-containing pool (1.2 M NaCl eluate) was subjected to HPLC either on a Mono-S HR5/5 (Pharmacia) cation-exchange column (Lobb et al., 1986a) or on a propylsilane (C3) column (5-μm particle size, 75 × 4.6 mm, Ultrapore RSPC, Beckman Instruments, Berkeley, CA) (Lobb & Fett, 1984; Lobb et al., 1986b). Material processed through the Mono-S cation-exchange chromatography step was desalted for chemical studies by reversed-phase HPLC (C3). The homogeneous mitogen stimulates half-maximal DNA synthesis in Balb/C 3T3 fibroblasts at a concentration of 250 pg/mL (Lobb et al., 1985b, 1986a,b).

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. The dried hydrolysates were subjected to amino acid analyses by the Picotag method (Waters Associates) (Bidlingmeyer et al., 1984; Cohen et al., 1985). Analyses after performic acid oxidation (Moore, 1963) were performed as described (Strydom et al., 1986).

Reduction and S-Sulfopropylation. Lyophilized HBGF-1 (4 nmol) was dissolved in 0.4 mL of 0.5 M Tris-HCl, 10 mM EDTA, and 6 M guanidine hydrochloride, pH 7.7. The solution was flushed with N_2, dithioerythritol added (final concentration, 6 mM), and reduction allowed to proceed for 45 min at 37 °C. 1,3-Propane sultone (in 50% 1-propanol, final concentration 40 mM) was added and alkylation allowed to occur for 2 h at 37 °C (Ruegg & Rudinger, 1977). The alkylated protein was desalted by reversed-phase HPLC (C3).

Proteolytic Digestion. Native HBGF-1 or RS-HBGF-1 (1–7 nmol) was dissolved in 0.5 M Tris-HCl, 10 mM EDTA, and 6 M guanidine hydrochloride, pH 7.7, and diluted with 9 volumes of 1% $NaHCO_3$ to give a final protein concentration of 4.5 nmol/mL. TLCK-treated chymotrypsin (2% w/w, Sigma Chemical Co.) or HPLC-purified trypsin (Titani et al., 1982; 5–7% w/w) was added and the mixture incubated for 21–24 h at 35 °C. The reaction was quenched by addition of 10% TFA (1.3% final concentration).

Peptide Mapping. Proteolytic digests were fractionated on a Synchropak RP-P column (Synchrom, Inc., Linden, IN, 250 × 4.5 mm) employing linear gradients with either a 0.1 M perchloric acid/0.1% phosphoric acid/acetonitrile (pH 2.5) solvent system or a 0.1% TFA/2-propanol/acetonitrile solvent system (Strydom et al., 1986). Peptides were further purified on an Altex Ultrapore IP column (Beckman Instruments, Inc., 250 × 4 mm, 5-μm particle size) with 0.1% TFA/acetonitrile solvent systems.

Sequencing Studies. Automated microsequencing was performed with a Beckman Model 890C sequencer updated to System 890 status as described (Strydom et al., 1985, 1986). *o*-Phthaladehyde 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). PTH-amino acids were identified and quantitated according to established procedures (Strydom et al., 1985). Serine was positively identified as PTH-Ser and its degradation products. Similarly, cysteine/cystine was unambiguously identified as a characteristic derivative of cysteine and its degradation products. PTH-Ser elutes in the PTH-amino acid separation system between His and Thr (retention time = 10.7 min) and is consistently seen at the position of serine residues in standard sequencer analyses. In contrast, the characteristic cysteine derivative, which is also seen consistently at the position of either cysteine or half-cystine residues, elutes midway between Tyr and Pro (retention time = 19.0 min). The degradation products associated with cysteine and serine are identical: a doublet before Tyr, a peak slightly earlier than Pro, and a peak slightly earlier than Trp. Thus, the identification of serine and cysteine and their differentiation from each other are based on the unequivocal presence of characteristic peaks in the PTH-amino acid separation system. The presence of a nonvolatile reducing agent (dithioerythritol) in the sequencer extractant (butyl chloride) causes reduction of cystine during the coupling reaction, allowing the identification of half-cystine as cysteine in sequencer degradations.

Other Techniques. Hydrazinolysis and hydroxylamine fragmentation were performed according to established procedures (Strydom et al., 1985). Cyanogen bromide fragmentation was performed in the sequencer cup as described (Strydom et al., 1986).

Results

Human brain HBGF-1 was purified to homogeneity by heparin affinity chromatography in combination with cation-exchange and/or reversed-phase HPLC. The protein migrates as a single band by SDS–PAGE with an apparent M_r of 17 000. The amino acid composition of highly purified human HBGF-1 is given in Table I.

N-Terminal Sequencing and Cyanogen Bromide Fragmentation. Edman degradation of human HBGF-1 (1.5 nmol) allowed assignment of the first 31 residues at the N-terminus [Figure 1a; supplementary material Tables I and II (see paragraph at end of paper regarding supplementary material)]. Two major sequences were observed; the second sequence begins with a deaminated Asn-2. At cycle 16, the sequencer-derived products of cysteine/cystine were clearly identified. At cycle 31, phenyl isocyanate was substituted for phenyl isothiocyanate (Boosman, 1980). This procedure blocks any free amino groups, giving a phenylurea derivative that does not undergo Edman degradation. The blocked protein was then subjected to cyanogen bromide cleavage in the sequencer cup at the single methionine residue as described previously for bovine HBGF-1 (Strydom et al., 1986). The cyanogen bromide fragment CNBr-2 with an unblocked α-amino group was then subjected to Edman degradation through 20 cycles (Figure 1c; Table II). The sequencer derived products of

Table I: Amino Acid Compositions of Human Brain HBGF-1 and Its Fragments[a]

	HC-1[c]	HC-2	HC-3	HC-4	HC-6b	HC-8	HC-10a	HC-10b	HC-11	HC-13[c]
Asp		0.61	0.88 (1)			1.09 (1)	1.32 (1)	1.05 (1)	1.83 (2)	4.36 (4)
Glu	1.98 (2)	2.01 (2)	3.01 (3)		1.14 (1)		3.29 (3)	1.46 (1)	0.23	2.47 (3)
Ser	1.04 (1)	2.16 (2)	0.40			1.94 (2)	0.48	1.38 (1)	0.46	1.74 (1)
Gly	1.20 (1)	2.53 (1)[b]	0.47		1.52 (1)	0.32	0.84	0.59	1.45 (1)	3.42 (2)
His			0.94 (1)				0.96 (1)	1.10 (1)	0.10	0.81 (1)
Arg			0.94 (1)				0.83 (1)			3.10 (3)
Thr	1.79 (2)								0.89 (1)	2.36 (2)
Ala		1.42 (1)			1.13 (1)		0.28	1.20 (1)	1.25 (1)	0.51
Pro	0.25	0.35		1.23 (1)		2.67 (2)	0.27			1.85 (1)
Tyr	0.72 (1)	1.00 (1)	0.73 (1)	0.91 (1)			1.06 (1)		0.97 (1)	
Val		1.95 (2)				0.97 (1)				0.98 (1)
Met									0.81 (1)	
Ile	0.81 (1)				0.84 (1)			1.07 (1)		2.04 (2)
Leu	0.22		1.06 (1)	2.29 (2)	0.99 (1)	2.05 (2)	2.21 (2)		2.87 (3)	3.81 (4)
Phe					1.04 (1)	0.90 (1)	0.98 (1)	0.93 (1)		0.37
Trp								(1)		
Lys	1.01 (1)			2.73 (3)	0.72 (1)			2.60 (3)		
Cys[e]		0[g]								
pmol analyzed	42	25	15	29	25	25	30	25	30	20
sequence position	56–64	47–55	87–94	9–15	126–132	132–140	85–94	98–108	65–74	23–46

	HT-1b	HT-3	HT-4[c]	HT-8a	HT-8b	HT-13	HT-15	HT-18b	human brain HBGF-1 HY-2	from sequence[d]	amino acid analysis
Asp		1.74 (2)	1.87 (2)	0.91 (1)	0.89 (1)	2.20 (2)	1.17 (1)	4.97 (5)	1.30 (1)	15	14.3
Glu	0.99 (1)	0.13	2.26 (2)	0.15	1.01 (1)	4.61 (5)	0.27	7.88 (8)	1.37 (1)	15	15.9
Ser	0.18	0.31	1.54 (1)	1.18 (1)	0.52	3.54 (3)	2.09 (2)	3.85 (3)	2.63 (3)	10	9.4
Gly	1.13 (1)	2.00 (2)	1.07	2.27 (2)	1.49 (1)	3.17 (1)	0.69	4.21 (3)	3.25 (3)	13	14.2
His	0.90 (1)		1.00 (1)	0.84 (1)	0.73 (1)	0.74 (1)		0.88 (1)	0.65 (1)	5	4.7
Arg		1.13 (1)	0.12	0.98 (1)		1.08 (1)		1.10 (1)	1.65 (2)	6	6.0
Thr	0.98 (1)	1.96 (2)	1.07 (1)			0.39		4.78 (5)	0.95 (1)	8	7.9
Ala		0.18	0.37		1.02 (1)	1.21 (1)	1.11 (1)	1.13 (1)	1.17 (1)	4	4.4
Pro		1.12 (1)	0.19			0.22	1.99 (2)	1.26 (1)	2.46 (3)	8	8.3
Tyr	0.97 (1)	0.22	1.43 (2)	1.29 (1)		1.22 (1)		4.42 (4)	(1)[j]	8	7.3
Val		1.04 (1)	0.17		0.94 (1)	1.81 (2)	1.13 (1)	0.32	1.24 (1)	5	4.7
Met								1.28 (1)		1	0.8
Ile		0.96 (1)	1.12 (1)			1.64 (2)	1.03 (1)	1.13 (1)	1.03 (1)	5	4.1
Leu		1.06 (1)	1.08 (1)	3.04 (3)	1.03 (1)	2.10 (2)	2.99 (3)	6.04 (6)	2.80 (3)	17	16.9
Phe		0.14		1.05 (1)	0.89 (1)	0.35	1.08 (1)	1.33 (1)	1.02 (1)	5	4.0
Trp					(1)					1	(1)[h]
Lys	0.98 (1)		2.00 (2)		2.67 (3)	1.32 (1)		2.03 (2)	1.79 (2)	11	11.4
Cys				0.73 (1)				0.52 (1)	0.80 (1)[f]	3	2.6[f,i]
pmol analyzed	60	37	20	30	30	28	65	7.8	33		
sequence position	123–128	25–35	89–101	13–24	102–113	36–57	129–140	58–101	115–140		
total										140	137

[a] Relative molar amounts of amino acids are given. Chymotryptic (HC) and tryptic (HT) peptides are numbered according to their HPLC elution order (see Figure 2). The hydroxylamine cleavage fragment is designated HY-2. Unless otherwise noted, peptides were rechromatographed in volatile solvent systems prior to amino acid analysis. Numbers in parentheses indicate the expected number of residues from sequence. Unless otherwise noted, Trp and Cys analyses were not performed. [b] Analyses are not corrected for background quantities of Gly, Ser, and Glu, which are present at this level in most of the HPLC fractions. [c] Analysis performed on aliquot from initial HPLC fractionation. [d] See Figures 1 and 3. [e] Determined as *S*-(sulfopropyl)cysteine unless otherwise noted. [f] Determined as cysteic acid after performic acid oxidation. [g] No trace of cysteic acid found after performic acid oxidation. [h] Determined from Edman degradation of HC-10b. [i] Average of two determinations with 27–30 pmol of protein analyzed. [j] Performic acid oxidation for detection of cysteine results in destruction of tyrosine. Amino acid analysis of HY-2 without performic acid showed 1.05 residues of Tyr.

cysteine/cystine were identified at cycle 16.

Hydroxylamine Fragmentation. The presence of an Asn–Gly bond at residues 18–19 in human HBGF-1 (Figure 1a) suggested the use of hydroxylamine for cleavage of the polypeptide chain at this position. With bovine HBGF-1, hydroxylamine fragmentation identified two Asn–Gly bonds, at residues 18–19 and 114–115, respectively (Strydom et al., 1986). Thus, human HBGF-1 (2 nmol) was digested with 2 M hydroxylamine (pH 9), the fragments were fractionated by molecular sieve chromatography, and the lower molecular weight fraction was rechromatographed by reversed-phase HPLC, as described for the bovine protein (Strydom et al., 1986). The sequence of the single low molecular weight peptide isolated, HY-2, was determined through 18 cycles (Figure 1d; Table II). At cycle three, the sequencer-derived products of cysteine/cystine were unambiguously identified. Consistent with this assignment, amino acid analysis of HY-2 after performic acid oxidation showed the presence of 0.8 residue of cysteic acid (Table I). The isolation of HY-2 in good yield suggests that Cys-3 in HY-2 is not disulfide bonded. This peptide did not correspond to residues 1–18 at the amino terminal of human HBGF-1, indicating that it was derived from cleavage at a second Asn–Gly bond. On the basis of the strict specificity of hydroxylamine for cleavage only at Asn–Xxx bonds (Bornstein, 1969; Bornstein & Balian, 1977; Blodgett et al., 1985), Gly-1 in this sequence must be preceded by asparagine (Figure 1d).

The higher molecular weight fragment (HY-3) obtained from molecular sieve chromatography was sequenced through 12 cycles (including *o*-phthalaldehyde blockage at cycle 9).

Table II: Amino Acid Sequences of Human HBGF-1 Fragments Obtained by Edman Degradation

Peptide	Sequencer Results[a]
CNBr-2	Asp-Thr-Asp-Gly-Leu-Leu-Tyr-Gly-Ser-Gln 162 210 377 469 457 549 373 336 ++ 227
	Thr-Pro-Asn-Glu-Glu-Cys-Leu-Phe-Leu-Glu 33 90 87 88 119 30 120 28 95 29
HY-2[b]	Gly-Ser-Cys-Lys-Arg-Gly-Pro-Arg-Thr-His 184 36 + 70 35 47 48 21 + 40
	Tyr-Gly-Gln-Lys-Ala-Ile-Leu-(Phe) 45 60 67 24 67 7 29 +
HY-3[c]	(Gly + Phe)-(Gly + Asn)-(His + Leu)-(Phe + Pro)- 530 133 326 110 170 198 487 137
	(Leu + Pro)-(Arg + Gly)-(Ile + Asn)-(Leu + Tyr)- 566 90 272 159 440 85 450 165
	Pro-Asp-Gly-Thr 69 81 56 +
HC-1[d]	Ile-Lys-Ser-Thr-Glu-Thr-Gly-Gln-Tyr-nothing 324 200 241 26 146 28 178 121 197
HC-2	Ser-Ala-Glu-Ser-Val-Gly-Glu-Val-Tyr 233 541 296 91 407 228 330 317 197
HC-8	Phe-Leu-Pro-Leu-Pro-Val-Ser-Ser-Asp-nothing 342 361 146 275 92 256 15 130 70
HC-10a	Phe-Leu-Glu-Arg-Leu-Glu-Glu-Asn-His-Tyr 100 68 89 59 65 54 70 41 (+) 44
HC-10b	Ile-Ser-Lys-Lys-His-Ala-Glu-Lys-Asn-Trp-Phe 221 45 23 127 50 86 77 63 64 6 12
HC-11	Leu-Ala-Met-Asp-Thr-Asp-Gly-Leu-Leu-Tyr 214 154 71 116 (+) 47 105 43 61 80
HT-3	Ile-Leu-Pro-Asp-Gly-Thr-Val-Asp-Gly-Thr-Arg 107 195 80 116 91 (+) 130 57 142 (+) 15
HT-4	Leu-Glu-Glu-Asn-Xxx-Tyr-Asn-Thr-Tyr-Ile-Xxx-Lys-Lys 213 79 82 29 - 53 25 (+) 33 35 - 15 13
HT-8b	His-Ala-Glu-Lys-Asn-Trp-Phe-Val-Gly-Leu-Lys-Lys 48 277 316 106 160 50 62 94 70 87 60 40
HT-13'	Asp-Arg-Ser-Asp-Gln-His-Ile-Gln-Leu-Gln- 197 56 22 79 34 20 24 49 59 42
	Leu-Ser-Ala-Glu-Xxx-Val-Gly-Xxx-Xxx-Tyr-Xxx-Xxx 42 (+) 21 27 - 11 16 - - 6
HT-18b	Xxx-Thr-Glu-Thr-Gly-Gln-Tyr-Leu-Ala- - (+) 140 (+) 230 130 90 130 180
	Met-Asp 80 110

[a] The established sequence is given, with the yield of the PTH-amino acid at each cycle given below the residue, in picomoles. Thr was positively identified (+) by the presence of its characteristic degradation product detected at 313 nm. Likewise, Cys and Ser were positively identified (+) by their characteristic PTH derivatives (see Materials and Methods). [b] Sequencer analysis for a second preparation of HY-2 yielded the following sequence: Gly(49)-Ser(45)-Cys(18)-Lys-(27)-Arg(24). The residue preceding Gly-1 in this sequence was assigned as asparagine as described under Results. [c] After eight degradation cycles, the peptide was reacted in the sequencer cup with *o*-phthalaldehyde as described for bovine HBGF-1 (Strydom et al., 1986), before resuming sequencing. [d] No trace of Leu found at cycle 10.

Two major sequences were observed, one beginning at Gly-19 and the other beginning at the amino terminal (Phe-1) (Table II). The sequences identified were readily placed in regions of the N-terminal sequence (Figure 1a) previously determined by Edman degradation. Consistent with the *N*-terminal microheterogeneity discussed above, a minor sequence beginning with a deaminated Asn-2 was also observed. The presence of two major N-terminal sequences could be due to either partial fragmentation or the presence of a disulfide bond linking Cys-16 to HY-3, or both.

Proteolytic Cleavage. The sequences of these chemical cleavage fragments established a total of 69 residues. In order to obtain additional sequence information, tryptic and chymotryptic digests were performed on reduced and S-sulfopropylated HBGF-1 and native HBGF-1, respectively. The tryptic and chymotryptic peptides were fractionated by reversed-phase HPLC (Figure 2). Most peptides were further purified by a second reversed-phase HPLC step. The amino

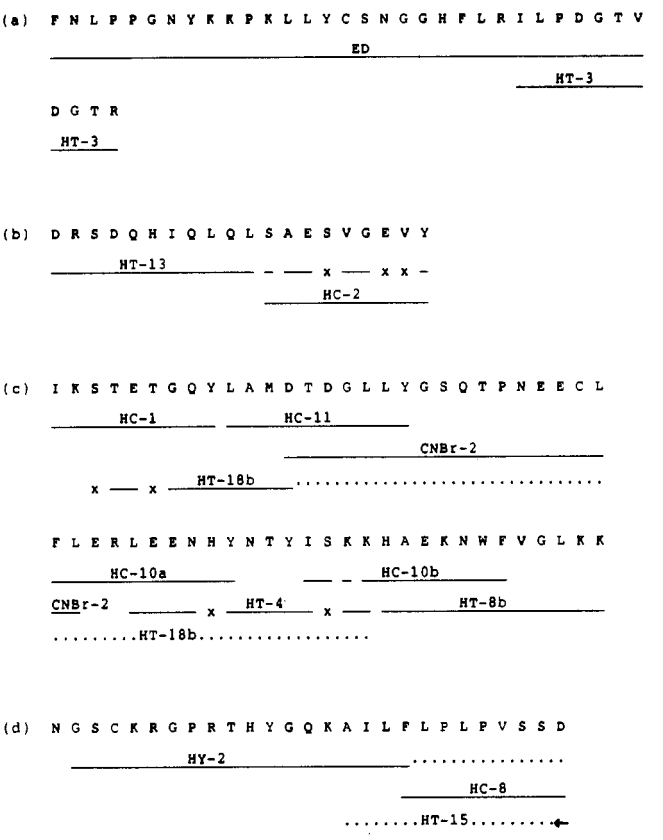

FIGURE 1: (a–d) Four nonoverlapping fragments of human HBGF-1 determined by Edman degradation. The extent of Edman degradation of cyanogen bromide (CNBr), hydroxylamine (HY), chymotryptic (HC), and tryptic (HT) digest products and of the intact molecule (ED) is indicated by solid lines. Dashed lines indicate weak assignments. Dotted lines indicate placement by amino acid composition. "X" indicates where no assignment was made. Chymotryptic and tryptic peptides are numbered as shown in Figure 2. The placement of peptide HT-15 is by amino acid composition, and its C-terminal residue was established as Asp by microhydrazinolysis.

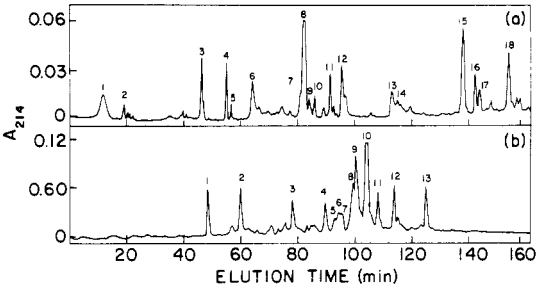

FIGURE 2: (a) Separation of tryptic peptides from human brain RS-HBGF-1 by HPLC on a Synchropak RP-P (C18) column. RS-HBGF-1 (1.5 nmol) was incubated with trypsin for 21 h at 35 °C and the reaction quenched with TFA before fractionation by HPLC. Peptide elution was accomplished with a linear gradient of 0–50% solvent B in 3 h at a flow rate of 0.8 mL/min. Solvent A was 0.1% TFA in water, and solvent B was 2-propanol/acetonitrile/water (3:2:2) containing 0.08% TFA. The fractions that contained significant quantities of amino acids after hydrolysis are numbered according to their order of elution. (b) Separation of chymotryptic peptides from human brain HBGF-1 by HPLC on a Synchropak RP-P (C18) column. HBGF-1 (7 nmol) was digested with chymotrypsin for 21 h at 35 °C and the reaction quenched with TFA. Peptides were eluted with a linear gradient from 0 to 50% solvent B in 3 h at a flow rate of 0.8 mL/min. Solvent A was 0.1 M perchloric acid/0.1% phosphoric acid (pH 2.5), and solvent B was 75% acetonitrile and 25% solvent A.

acid compositions of some of the peptides obtained in pure form are given in Table I. The analyses are not corrected for background quantities of Ser, Gly, or Glu, which were found

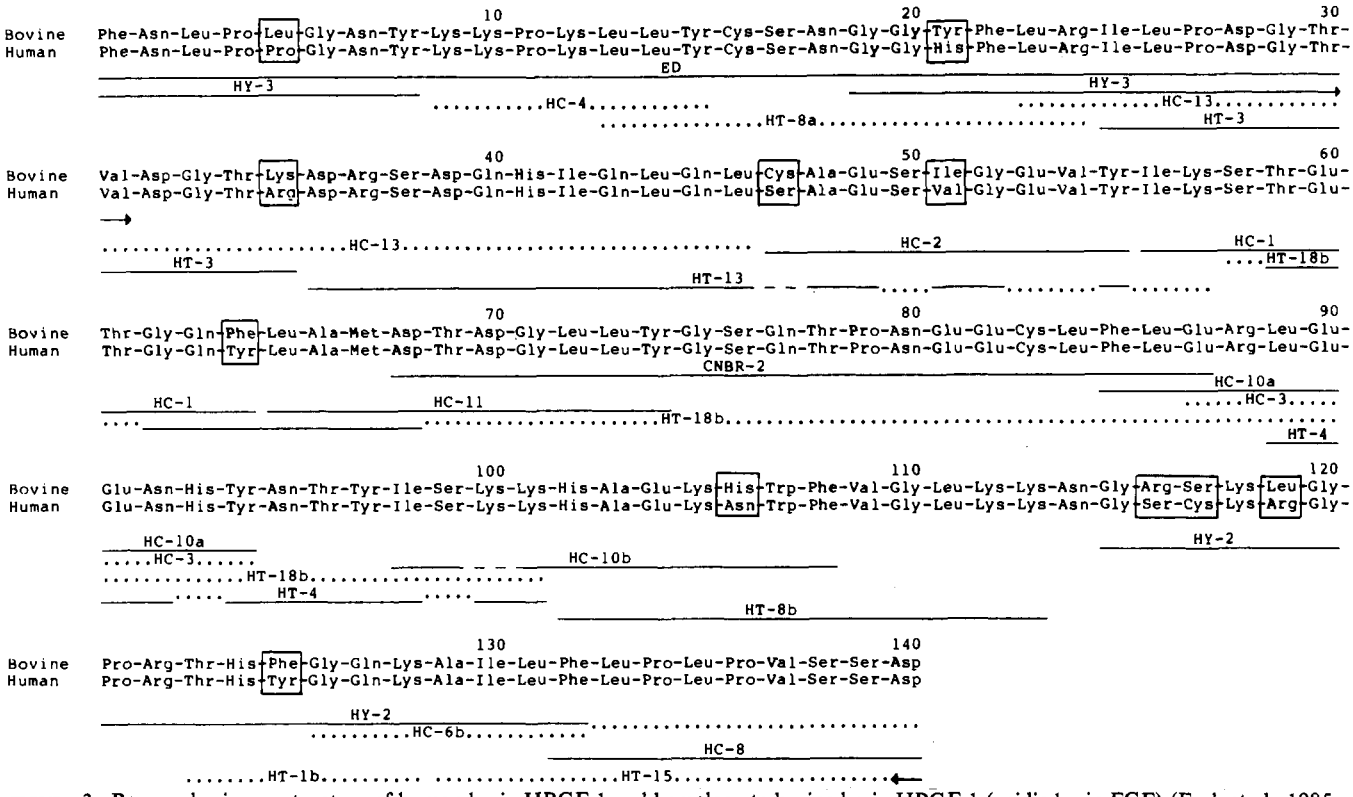

FIGURE 3: Proposed primary structure of human brain HBGF-1 and homology to bovine brain HBGF-1 (acidic brain FGF) (Esch et al., 1985a; Gimenez-Gallego et al., 1985; Strydom et al., 1986). The extent of Edman degradation of cyanogen bromide (CNBr), hydroxylamine (HY), chymotryptic (HC), and tryptic (HT) digest products and of the intact molecule (ED) is indicated by solid lines. Dashed lines indicate weak assignments, while dotted lines indicate placement by amino acid composition (Table I). Chymotryptic and tryptic peptides are numbered as shown in Figure 2. Boxed regions indicate nonidentical residues.

at this level in most samples isolated by HPLC. The sequences of peptides HC-1, HC-2, HC-8, HC-10a, HC-10b, HC-11, HT-3, and HT-8b were firmly established by Edman degradation while partial sequences of HT-4, HT-13, and HT-18b were obtained (Table II). In addition, peptides HC-4, HC-3, HC-6b, HT-8a, HT-1b, and HT-15 could be placed directly into regions of known sequence on the basis of their amino acid compositions (see below).

C-Terminal Analysis. The sequence of the chymotryptic peptide HC-8 terminates with aspartic acid (Figure 1d, Table II), suggesting that this peptide occupies the C-terminal of human HBGF-1. The amino acid composition of tryptic peptide HT-15 (Table I), which contains neither Lys nor Arg, indicates that is spans HY-2 and HC-8. Microhydrazinolysis of HT-15 (200 pmol) established its C-terminal residue as aspartic acid. These findings indicate that segment 4 (Figure 1d) represents the C-terminal of human HBGF-1.

Alignment of Fragments. The sequence data detailed above established the following four nonoverlapping segments of human HBGF-1: (1) the 35-residue segment defined by HT-3 and the N-terminal 31-residue sequence (Figure 1a); (2) the 20-residue segment defined by HT-13 and HC-2 (Figure 1b); (3) the 58-residue segment defined by HC-1, HT-18b, HC-11, CNBr-2, HC-10a, HT-4, HC-10b, and HT-8b (Figure 1c); (4) the 27-residue segment defined by HY-2 and HC-8 (Figure 1d). The sum of the amino acids of these four segments is in excellent agreement with the amino acid composition of HBGF-1 determined experimentally (Table I). This suggests that, jointly, these sequences account for the entire HBGF-1 molecule.

The data indicate that segment 1 and segment 4 represent the N- and C-termini, respectively (Figure 3). Segments 2 and 3 can be aligned in that order on the basis of the partial sequence and amino acid composition of the pure tryptic peptide HT-13 (Figure 3, Tables I and II). First, on the basis of the specificity of trypsin and the composition and partial sequence of HT-13, this peptide must terminate in lysine. Second, all the lysines in HBGF-1 have been placed in the four segments (Table I, Figure 1). Thus, by inspection of the peptide sequence and composition data, HT-13 must overlap peptide HC-1 in segment 3. Third, the specificity of chymotrypsin in conjunction with the amino acid composition of HT-13 argues strongly that there are no additional residues between segments 2 and 3. Thus, segments 1–4 can be aligned in that order. Consistent with this alignment, the amino acid composition of chymotryptic peptide HC-13 (Table I) indicates that it spans segments 1 and 2 (Figure 3). The resultant alignment of peptides HT-3–HT-13 and peptides HC-13–HC-2 is in agreement with the established proteolytic specificities of trypsin and chymotrypsin. A peptide spanning segments 3 and 4 (Figure 1) could not be identified in two digests with trypsin and chymotrypsin for 24 h at 35 °C. We were also unable to identify peptides spanning this region when bovine brain HBGF-1 was exhaustively digested (24 h, 37 °C) with trypsin or chymotrypsin (Strydom et al., 1986). A chymotryptic peptide spanning this region in the bovine protein was only identified when a limited (3-h) digest was performed. The reasons for this are unclear.

Homology to Bovine Acidic Brain FGF. Comparison of the sequences of the four aligned segments describing human HBGF-1 with the complete amino acid sequence of bovine acidic brain FGF (Gimenez-Gallego et al., 1985; Esch et al., 1985; Strydom et al., 1986) shows no less than 92% identity (Figure 3). There are neither insertions nor deletions, and there are no N-terminal or C-terminal extensions. Thus, on the basis of the amino acid composition of HBGF-1 and of its segments, the established specificities of chymotrypsin and trypsin, and the extremely high homology between the two

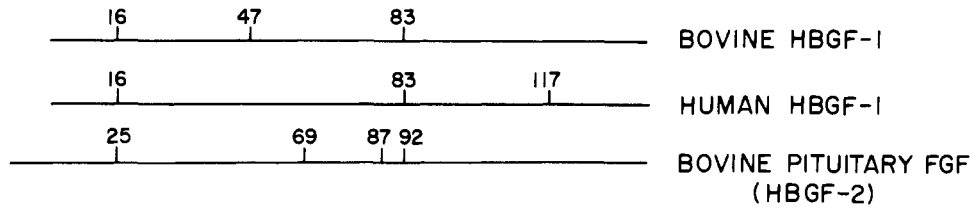

FIGURE 4: Alignment of half-cystine residues in human brain HBGF-1, bovine acidic brain FGF (HBGF-1), and bovine pituitary FGF (HBGF-2). On the basis of homology, Cys-16 and Cys-83 in the HBGF-1's correspond to Cys-25 and Cys-92 in pituitary FGF, respectively.

mitogens, the sequence shown in Figure 3 likely represents the complete primary structure of human HBGF-1.

DISCUSSION

Class 1 HBGF's have been purified to homogeneity from several neural tissues including brain, hypothalamus, and retina and are mitogenic for a wide variety of mesoderm- and neuroectoderm-derived cells in vitro [for review, see Lobb et al. (1986a)]. In addition, homogeneous class 1 HBGF's from bovine brain induce neovascularization in vivo (Lobb et al., 1985a; Thomas et al., 1985). Both the mitogenic and angiogenic activities of class 1 HBGF's are enhanced by heparin (Lobb et al., 1985a, 1986a, Thomas et al., 1985; Schreiber et al., 1985), a characteristic that distinguishes them from class 2 HBGF's.

The physical characteristics (molecular weight, isoelectric point, chromatographic properties) of the class 1 HBGF's in bovine neural tissue suggest that these proteins are closely related if not identical molecules (Lobb et al., 1986a,b). Moreover, the amino acid compositions of human and bovine brain HBGF-1 (Lobb et al., 1985b, 1986b) are very similar, implying closely related structures. Thus, the strategy used to solve the sequence of bovine brain HBGF-1 (Strydom et al., 1986) was applied directly to the human protein.

The presence of a single methionine residue in human HBGF-1 (Table I) provided an opportunity for sequencing of the intact protein and cyanogen bromide fragment in a single experiment, a strategy that proved effective with human lymphotoxin (Aggarwal et al., 1985). For HBGF-1, this strategy allowed the sequence of both the amino-terminal 31 residues and the 20 residues following the single methionine to be determined. Thus, 36% of the sequence was established in one experiment utilizing only 1.5 nmol of human HBGF-1.

Sequencing of HBGF-1 fragments generated by chemical cleavage with hydroxylamine and proteolytic cleavage with either trypsin or chymotrypsin confirmed and extended these sequences. The primary structure of human HBGF-1 could be described in four nonoverlapping segments (Figure 1). These segments could be aligned into a presumptive complete sequence, which was corroborated by its extensive homology to bovine acidic brain FGF.

Figure 3 compares the two sequences which show 92% identity. The longest stretch of sequence identity occurs between residues 65 and 105, which includes a highly acidic cluster (residues 80–91) strongly conserved between class 1 and class 2 HBGF's (Esch et al., 1985a; Strydom et al., 1986). Indeed, only 11 amino acids differ between human HBGF-1 and bovine HBGF-1 (Figure 3). Eight of these changes (positions 21, 35, 47, 51, 64, 106, 117, and 125) are conservative, resulting in an overall sequence similarity of 98%.

The most striking difference between human HBGF-1 and bovine HBGF-1 relates to the placement of the three half-cystine residues. Surprisingly, exchanges of half-cystine for serine and serine for half-cystine have occurred at positions 47 and 117, respectively (Figure 3). Thus, while both proteins contain three half-cystine residues, only two of the three are conserved (positions 16 and 83). We have suggested the existence of a disulfide bond between Cys-16 and Cys-83 in bovine HBGF-1 on the basis of chemical evidence and homology to bovine pituitary FGF (Strydom et al., 1986). Hydroxylamine fragmentation of human HBGF-1 is also consistent with a disulfide bond between Cys-16 and Cys-83, leaving Cys-117 as a free sulfhydryl, although direct evidence is lacking. Figure 4 shows the alignment of these half-cystine residues in the HBGF-1's and compares them with the homologous protein bovine pituitary FGF, a class 2 HBGF (Esch et al., 1985b). The half-cystines at positions 16 and 83 in the class 1 HBGF's are conserved in pituitary FGF at positions 25 and 92, respectively.

Since cysteinyl residues are quite rare in proteins, the Cys/Ser interchanges observed suggest a specific role for a cysteinyl residue in the function of these mitogens. While the cysteinyl residues in bovine and human HBGF-1 are found in different positions in the peptide chain, the tertiary structure of the protein could place these residues in a similar critical spatial arrangement. Alternatively, the role of the cysteinyl residue may be as a carrier of a small molecule of importance for mitogenic activity. In the case of bovine pituitary FGF, two of the four half-cystine residues show evidence of linkage via disulfides to a small molecule (Esch et al., 1985b). This interaction could be with free cysteine or with a cysteine-containing molecule such as glutathione. Clearly, the role of cysteine in HBGF's deserves further study.

The Edman degradation of human HBGF-1 consistently showed two N-terminal sequences in approximately equal quantities, the second beginning with a deaminated Asn-2. This N-terminal microheterogeneity differs from that exhibited by bovine brain HBGF-1 (Thomas et al., 1985; Strydom et al., 1986) where a form beginning with Asn-7 is obtained in varying amounts ranging from 40 to 60%. Bovine pituitary FGF also exhibits N-terminal microheterogeneity with a form beginning with His-16 (Esch et al., 1985a), the position homologous to Asn-7 in bovine HBGF-1.

A further difference between human and bovine HBGF-1 involves the presence of a potential glycosylation site in the human protein (Asn-Gly-Ser, residues 114–116) which, due to the Arg/Ser interchange at position 116, is not found in bovine HBGF-1 (Figure 3). The carbohydrate content of human HBGF-1 is not known. However, the similar molecular weights of human and bovine HBGF-1 (Lobb et al., 1985b, 1986b) indicate that the human mitogen is not extensively glycosylated.

We have suggested that two regions in bovine brain HBGF-1 containing clusters of basic residues (Lys-9 to Lys-12 and Lys-100 to Arg-122) represent likely heparin-binding sites (Strydom et al., 1986). Residues 9–12 are fully conserved between the human and bovine proteins. However, in the region from 100 to 122, four substitutions occur (Figure 3). Since three of these substitutions occur between residues 115 and 119, these residues may not play an essential role in heparin binding.

The high degree of homology observed between human and

bovine brain HBGF-1's indicates a very low rate of accepted point mutation rate of about 6.5 mutations per 100 residues per 100 million years (McLaughlin & Dayhoff, 1972). This mutation rate is similar to that observed with mammalian cytochrome *c*'s, which are very slowly evolving proteins (Dayhoff et al., 1972). Such rigorous evolutionary constraints on structure are usually found with proteins involved in critical interactions with more than one other macromolecule as is found in, for example, the cytochrome *c*/cytochrome oxidase/reductase system. Thus, the high sequence identity within class 1 HBGF's implies that most of the surface structure of the molecule has functional significance. This would be consistent with the involvement of a ternary mitogen/receptor/heparin complex in HBGF function. Alternatively, different structural domains in HBGF-1 may regulate different functions, such as mitogenesis and chemotaxis, as has been demonstrated for platelet-derived growth factor (Williams et al., 1983).

In conclusion, the amino acid sequence of human brain HBGF-1 in conjunction with that of bovine brain HBGF-1 provides further insight into the structural requirements for HBGF function and serves as a basis for detailed structure/function studies.

Added in Proof

After submission of the manuscript, the amino-terminal sequences of both human acidic and human basic brain FGF's were published by Giminez-Gallego et al. (1986). The 32-residue amino-terminal sequence of human acidic brain FGF is identical with that given here (Figures 1 and 3).

Acknowledgments

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Supplementary Material Available

Two tables containing yields of PTH-amino acids by Edman degradation (2 pages). Ordering information is given on any current masthead page.

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