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NH_2-Terminal amino acid sequence and peptide mapping of purified human β-lipotropin: Comparison with previously proposed sequences

(spinning cup sequence analysis/reverse-phase high performance liquid chromatography/cyanogen bromide cleavage)

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ABSTRACT β-Lipotropin was purified from human pituitary glands to a purity of greater than 90%. The amino acid compositions of β-lipotropin and its three cyanogen bromide cleavage peptide fragments were in agreement with the structure proposed by Li and Chung [Li, C. H. & Chung, D. (1981) *Int. J. Pept. Protein Res.* 17, 131–142]. However, the amino acid sequence of its NH_2-terminal 46 amino acid residues established here differs both from the sequence derived from the direct sequence analysis of the peptide reported by Li and Chung and from that predicted on the basis of the nucleotide sequence of the human pro-opiolipomelanocortin gene proposed by Chang *et al.* [Chang, A. C. Y., Cochet, M. & Cohen, S. W. (1980) *Proc. Natl. Acad. Sci. USA* 77, 4890–4894] but agrees with the structure recently derived by direct sequence analysis by Hsi *et al.* [Hsi, K. L., Seidah, N. G., Lu, C. L. & Chrétien, M. (1981) *Biochem. Biophys. Res. Commun.* 103, 1329–1335] and predicted on the basis of nucleotide sequence analysis by Takahashi *et al.* [Takahashi, H., Teranishi, Y., Nakanishi, S. & Numa, S. (1981) *FEBS Lett.* 135, 97–102]. These discrepancies, found from residues 9 to 25 of β-lipotropin, could result from pro-opiolipomelanocortin gene polymorphism, from the existence of multiple genes for pro-opiolipomelanocortin, or, more probably, from minor errors in nucleotide and amino acid sequence analyses.

Pro-opiolipomelanocortin (proOLMC) is synthesized in the pituitary gland as the common precursor of β-lipotropin (βLPH), adrenocorticotropin (ACTH), and pro-γ-melanotropin (proγMSH) (1), each of which is a precursor to other polypeptides that are known or presumed to exhibit specific biologic activities. βLPH contains the structures of β-endorphin (βEND) and γLPH which is itself a precursor of βMSH (1).

Recently, the nucleotide sequence of human genomic DNA corresponding to most of proOLMC mRNA was determined by Chang *et al.* (2). The amino acid sequence predicted for proOLMC agreed with the amino acid sequences obtained directly for human ACTH (3–5), the COOH-terminal part of human βLPH corresponding to βMSH and βEND (6, 7), and human proγMSH-(1–76) (8). Human proγMSH-(79–108) deviated from the primary structure predicted by nucleotide sequence analysis by only one residue (9). However, the predicted amino acid sequence of the NH_2-terminal part of βLPH differed significantly (by 13 residues) from the sequence determined directly (7). Because the genomic DNA was derived from one individual, it was suggested (2) that the discrepancies might reflect proOLMC gene polymorphism or spontaneous nucleotide base substitutions during cloning. Another explanation would be the existence of multiple proOLMC genes (10–15). Clarification of these discrepancies could be important for understanding the production of proOLMC peptides by normal and neoplastic tissues (16–19).

Because Li and Chung (20) had proposed two revisions of the amino acid sequence of human βLPH after the report by Chang *et al.* (2), it was unclear how much significance should be attached to the remaining discrepancies between the respective sequences. Therefore, it seemed desirable to reinvestigate the NH_2-terminal structure of human βLPH with an analytical approach different from that of Li and Chung (7, 20) who applied the dansyl Edman technique to enzymatic fragments of βLPH. βLPH was subjected to sequence analysis in a Beckman modified spinning cup sequencer without prior enzymatic or chemical fragmentation. In addition, the amino acid compositions of βLPH and its three CNBr cleavage peptides were determined.

MATERIALS AND METHODS

Isolation of Human βLPH. One thousand freshly frozen human pituitary glands (lot HP72-17) were obtained from The National Pituitary Agency (National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases). In the purification scheme (see Table 1), βLPH was followed by radioimmunoassay with either antiserum G106, which is specific for βLPH-(35–56)‡ (21), or R2489, which is specific for βLPH-(72–89)‡ (ref. 22; unpublished data), or both. The frozen glands (combined weight, 447.5 g) were divided into six aliquots, homogenized at 20°C in glacial acetic acid (10 ml/g of tissue) in a Waring Blendor, and centrifuged at 50,000 × g for 20 min at 4°C. The supernates were diluted with 10 vol of water, adjusted to pH 3.3, applied to an Amberlite CG-50 column (2.6 × 70 cm) equilibrated with 5% acetic acid, and eluted with 60% acetic acid. The eluates were lyophilized, extracted with 0.1 M ammonium formate/0.2 M acetic acid, and applied to a Sephadex G-75 (fine) column (5 × 90 cm) equilibrated and developed with 0.1 M ammonium formate/0.2 M acetic acid. Fractions that contained βLPH were pooled, lyophilized, and further purified on a CM-cellulose column (1.6 × 5 cm) equilibrated with 0.01 M ammonium acetate buffer (pH 4.6) and eluted with an exponential ammonium acetate gradient (0.01 M at pH 4.6 to 0.1 M at pH 6.7). Fractions containing βLPH were pooled, lyophilized, redissolved in 0.01 M ammonium acetate buffer (pH 4.6), applied to a CM-cellulose column (1.6 × 5 cm) equilibrated with the same buffer, and eluted with a linear ammonium acetate gradient (0.03 M to 0.06 M, pH 5.5). Dansyl chloride end group analysis (23) was performed on this material.

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Abbreviations: proOLMC, pro-opiolipomelanocortin; βLPH, β-lipotropin; ACTH, adrenocorticotropin; MSH, melanotropin; βEND, β-endorphin; >PhNCS, phenylthiohydantoin.

‡ Based on an 89-amino acid βLPH molecule as proposed in ref. 20 and this study.

Table 1. Purification of βLPH from 1,000 human pituitaries

		Product			
Step	Procedure	Protein,* g	βLPH,† g	Specific activity‡	Recovery, %
1	Acetic acid extraction	49.7	0.345	0.007	100.0
2	Ion exchange (Amberlite CG-50)	17.1	0.341	0.020	98.8
3	Gel filtration (Sephadex G-75)	0.312	0.135	0.433	39.1
4	Ion exchange (CM-cellulose)	0.0615	0.063	1.024	18.3
5	Ion exchange (CM-cellulose)	0.0425	0.043	1.011	12.5

* Determined by the method of Lowry *et al.* (32) in step 1, dry weight in step 2, and absorbance at 280 nm in steps 3–5.
† Determined by radioimmunoassay.
‡ Calculated as the ratio (wt/wt) of immunoreactive βLPH to protein content.

Reverse-phase HPLC was used for final purification of βLPH. The HPLC apparatus consisted of two Waters model 6000A pumps, a model 660 gradient programmer, a model 440 absorbance detector, and a model 450 variable-wavelength detector. The solvent systems of Bennett *et al.* (24) were used. βLPH (1 mg) from the CM-cellulose column was applied to a Waters μBondapak CN column (0.39 × 30 cm) and eluted with a linear gradient (20 min, 30% to 35% acetonitrile in 0.1% F_3CCOOH). The βLPH was reapplied to the same column and eluted with a linear gradient (10 min, 42% to 48% acetonitrile in 0.01 M F_7C_3COOH). This material was used for CNBr cleavage (25) and sequence analysis.

CNBr Cleavage and Peptide Fragment Purification. CNBr (20 mg/ml) was dissolved in 75% F_3CCOOH saturated with argon for 1 hr just prior to use. Either βLPH or synthetic βEND, highly purified by reverse-phase HPLC from 1 mg of peptide, was dissolved in 200 μl of fresh CNBr solution, incubated at 22°C for 24 hr under argon, and diluted with 1 ml of water. The mixture was applied to a μBondapak C_{18} column (0.39 × 30 cm) and eluted with a linear gradient (60 min, 12% to 80% acetonitrile in 0.1% F_3CCOOH for βLPH or 12% to 40% acetonitrile in 0.1% F_3CCOOH for βEND). An aliquot of βLPH not treated with CNBr was applied to and eluted from the same column with the same gradient. Peptides were monitored by absorbance at 210 and 280 nm. Peptides that were not completely resolved were rechromatographed. Aliquots of the synthetic βEND fragment and the βLPH fragment (CNBr III) with similar retention times were mixed, applied to the C_{18} column, and eluted with a linear gradient (20 min, 28% to 40% acetonitrile in 0.1% F_3CCOOH).

Amino Acid Analysis. Amino acid compositions of βLPH and its CNBr fragments were determined (26) after hydrolysis with 4 M methanesulfonic acid/0.2% tryptamine (27) (24 hr at 110°C or 3 hr at 140°C) or with constant boiling HCl containing 3 μl of 2-mercaptoethanol per ml (110°C for 24, 48, and 72 hr). Norleucine was added before hydrolysis as internal standard. Coefficient of variation was <10%.

Spinning Cup Sequence Analysis. Sequence analysis was performed by Edman degradation in a Wittmann-Liebold (28, 29) modified Beckman 890C spinning cup sequencer (27, 30, 31). Extensively purified Polybrene was added to the cup and precycled prior to peptide application. The phenylthiohydantoin (>PhNCS) derivatives of all frequently occurring amino acids, automatically generated in a converting flask, were identified and quantified by reverse-phase HPLC (31). Usually, small amounts of asparagine and glutamine were desamidated during Edman degradation under these conditions. The desamidated derivatives, >PhNCS-aspartic acid and >PhNCS-glutamic acid, represented approximately 5–15% of the amidated forms. Repetitive yields of stable >PhNCS amino acids were >93%. Carryover was usually <10%.

RESULTS

A total of 43 mg of βLPH was obtained from 1,000 human pituitaries, as determined by radioimmunoassay (Table 1). The overall yield (43 μg of βLPH per pituitary gland) was comparable to that (34 μg of βLPH per pituitary gland) reported by Li and Chung (20). The immunologic characteristics of this preparation have been described (21, 33); its specific activity was estimated to be 1 μg of immunoreactive βLPH per μg of protein (Table 1). End-group determination (23) performed on this preparation yielded glutamic acid (or glutamine) as the predominant amino acid, but reverse-phase HPLC revealed the presence of contaminating peptides. Consequently, aliquots of this βLPH preparation were subjected to further purification by reverse-phase HPLC.

Amino acid analysis of βLPH purified by reverse-phase HPLC (Fig. 1*A*) was performed after hydrolysis with aqueous methanesulfonic acid or hydrochloric acid. Ion exchange chromatography of the methanesulfonic acid hydrolysate established that the purified peptide contained all frequently occur-

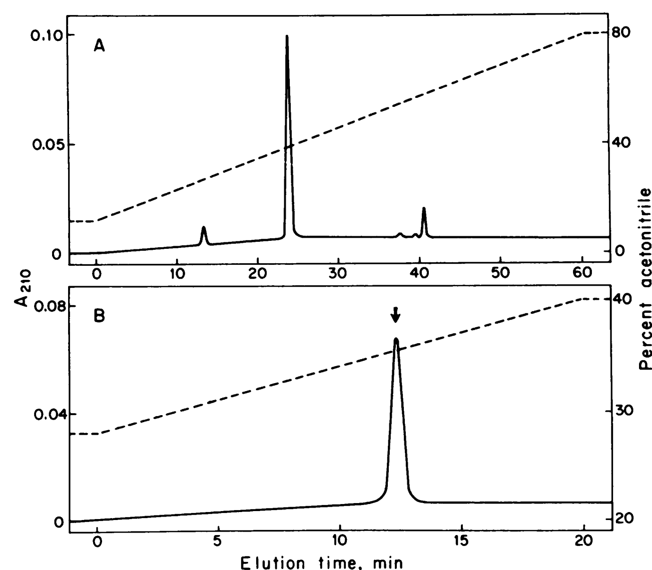

FIG. 1. Reverse-phase HPLC of highly purified βLPH (*A*) and of a 1:1 mixture of CNBr fragment III of βLPH and the COOH-terminal CNBr fragment of synthetic human βEND (*B*). (*A*) βLPH (0.5 μg) purified by reverse-phase HPLC was applied to a μBondapak C_{18} column (0.39 × 30 cm) and eluted with increasing concentrations of acetonitrile in 0.1% F_3CCOOH. (*B*) The mixture of CNBr cleavage products (0.5 μg of each) was eluted from the same column with the same buffer system. The four minor peaks in *A* are artifacts that also appeared when solvent alone was applied to the column. Arrow, elution time of CNBr III chromatographed in a separate run under identical conditions; ——, absorbance at 210 nm; ----, percent acetonitrile in elution gradient.

Table 2. Amino acid composition of human βLPH

Amino acid	Acid hydrolysis*	Amino acid sequence analysis†	Nucleotide sequence analysis‡
Asx	8.6 (9)	9	7
Thr	3.7 (4)	4	5
Ser	3.6 (4)	4	4
Glx	10.9 (11)	11	11
Pro	6.2 (6)	6	7
Gly	11.0 (11)	11	11
Ala	8.1 (8)	8	7
Val	2.0 (2)	2	2
Met	1.7 (2)	2	2
Ile	1.7 (2)	2	2
Leu	7.1 (7)	7	7
Tyr	3.0 (3)	3	3
Phe	3.0 (3)	3	3
Lys	9.0 (9)	9	9
His	2.1 (2)	2	2
Trp	0.8 (1)	1	1
Arg	4.9 (5)	5	5

* Aliquots of the final purification product (5 μg of protein) were hydrolyzed (110°C for 24, 48, and 72 hr) with constant boiling HCl containing 3 μl of 2-mercaptoethanol per ml and norleucine as internal standard. The hydrolysates were subjected to cation exchange chromatography with sodium citrate buffers used for elution. Normalized amino acid concentrations were extrapolated to the starting time of hydrolysis. Amino acid ratios are presented; the nearest integer is given in parentheses.

† Derived from the revised structure proposal (20).

‡ Derived from the amino acid sequence predicted by nucleotide sequence analysis (2).

ring amino acids except cysteine (Table 2). The amino acid composition of βLPH did not deviate from the revised data presented by Li and Chung (20) but did deviate by 5 residues from the composition predicted on the basis of nucleotide sequence analysis data (2) and by 7–10 residues from amino acid compositions described earlier (6, 34, 35).

CNBr cleaved βLPH at its two methionine residues to give three fragments, which were purified by reverse-phase HPLC. The COOH-terminal fragment, CNBr III, co-eluted with the corresponding CNBr cleavage fragment of synthetic human βEND (Fig. 1*B*). The amino acid compositions of the central fragment (CNBr II) and CNBr III (Table 3) did not deviate significantly from those proposed by Li and Chung (20), Cseh *et al.* (6), and Chang *et al.* (2). However, the composition of the NH_2-terminal fragment, CNBr I, which was in agreement with the revised data of Li and Chung (20), deviated significantly from the composition predicted by Chang *et al.* (2) on the basis of the proOLMC nucleotide sequence (Table 2).

Sequence analysis was performed in two separate sequencer experiments. In the first experiment, 4 nmol of βLPH was preincubated with 3-sulfophenylisothiocyanate before addition to the precycled Polybrene in the spinning cup to improve the binding of the peptide to the positively charged Polybrene film. Edman degradation was then accomplished with a standard sequencer program (31). The NH_2-terminal 39 residues of βLPH, identified as the predominant polypeptide of the analyzed fraction on the basis of >PhNCS amino acid yields, were determined in this experiment. Due to interference by coupling products of 3-sulfophenylisothiocyanate in the reverse-phase HPLC identification of >PhNCS amino acids, it was impossible to detect and quantify smaller amounts of contaminating peptides. In the second experiment, 4 nmol of peptide was applied to the cup, but the initial coupling to 3-sulfophenylisothiocyanate was omitted and the cleavage conditions were varied. The peptide was subjected to two cleavage reactions in cycles 13, 16, and 42 in order to facilitate proline release. All other cycles were performed under milder cleavage conditions than in the first sequencer experiment (cleavage time was 300 sec instead of 400 sec). This experiment confirmed that βLPH was the main polypeptide in the analyzed fraction. Contaminating peptides

Table 3. Amino acid composition of human βLPH CNBr fragments

Amino acid	CNBr I			CNBr II		CNBr III	
	Acid hydrolysis*	Sequence analysis A†‡	Sequence analysis B§	Acid hydrolysis*	Sequence analysis‡§	Acid hydrolysis*	Sequence analysis‡§
Asx	5.5 (6)	6	4	0.9 (1)	1	1.7 (2)	2
Thr	0.9 (1)	1	2	0.0 (0)	0	2.8 (3)	3
Ser	0.9 (1)	1	1	0.9 (1)	1	1.8 (2)	2
Glx	6.7 (7)	7	7	1.2 (1)	1	3.0 (3)	3
Pro	3.3 (3)	3	4	2.0 (2)	2	1.1 (1)	1
Gly	7.0 (7)	7	7	3.0 (3)	3	1.2 (1)	1
Ala	6.4 (6)	6	5	0.3 (0)	0	2.1 (2)	2
Cys	0.0 (0)	0	0	0.0 (0)	0	0.0 (0)	0
Val	0.9 (1)	1	1	0.0 (0)	0	1.0 (1)	1
Met	0.0 (0)	0	0	0.0 (0)	0	0.0 (0)	0
Ile	0.0 (0)	0	0	0.0 (0)	0	1.5 (2)	2
Leu	4.9 (5)	5	5	0.0 (0)	0	2.1 (2)	2
Tyr	1.0 (1)	1	1	0.7 (1)	1	1.0 (1)	1
Phe	0.0 (0)	0	0	1.7 (2)	2	1.1 (1)	1
Lys	2.3 (2)	2	2	2.0 (2)	2	5.0 (5)	5
His	0.9 (1)	1	1	0.9 (1)	1	0.0 (0)	0
Trp	0.0 (0)	0	0	0.9 (1)	1	0.0 (0)	0
Arg	2.9 (3)	3	3	1.9 (2)	2	0.1 (0)	0

* Aliquots of CNBr fragments I, II, and III (0.3–4 μg of protein) were hydrolyzed (110°C for 24 hr) with constant boiling HCl containing 3 μl of 2-mercaptoethanol per ml and norleucine as internal standard. The hydrolysates were subjected to cation exchange chromatography with lithium citrate buffers used for elution. The hydrolysates of fragments I and II contained small amounts of homoserine. Amino acid ratios are presented; the nearest integer is given in parentheses.

† Data derived from sequence analysis performed in this study.

‡ Data derived from revised structure proposal (20).

§ Data predicted on the basis of nucleotide sequence analysis (2).

FIG. 2. Sequence analysis of human βLPH (4.0 nmol). The peptide was applied to the cup containing Polybrene (4 mg). One-third of each sequencer fraction, collected from the converting flask, was analyzed by reverse-phase HPLC. Total >PhNCS amino acid yields are shown.

represented <7% of the main component. These results, together with the data of end-group determinations, amino acid analysis, reverse-phase HPLC, and determination of the specific immunoreactivity, indicated a purity of βLPH of >90%.

The sequence of the NH_2-terminal 46 amino acid residues of βLPH was obtained in the second sequencer experiment (Fig. 2); all residues found in the first experiment were confirmed. The NH_2-terminal amino acid sequence of βLPH deviated from the sequences proposed by Li and Chung (20) and Chang *et al.* (2) (Fig. 3). The deviations from the sequence of Li and Chung (20) comprised five residues (residues 9, 14, 15, 16, and 21) but did not affect the amino acid composition of the peptide hydrolysate and thus were in agreement with the amino acid analysis data (Tables 2 and 3). The deviations from the sequence predicted by Chang *et al.* (2) were confined to seven residues (residues 18–21 and 23–25) and were consistent with the observed deviations (Tables 2 and 3) from the amino acid compositions predicted by Chang *et al.* (2).

DISCUSSION

Human pituitary βLPH was purified by ion exchange chromatography, gel filtration, and reverse-phase HPLC to a purity of >90%.

The COOH-terminal sequence of human βLPH, which includes the structures of βMSH and βEND, has been determined by sequence analysis in three different laboratories (2, 6, 7, 20). Accordingly, it was not surprising that amino acid compositions of the central and COOH-terminal CNBr fragments of βLPH and reverse-phase HPLC of the COOH-terminal CNBr fragment provided no evidence for deviation from the previously reported βLPH structure.

The entire NH_2-terminal amino acid sequence in question was unambiguously determined, without the need of chemical or enzymatic fragmentation, by Edman degradation in a Wittmann-Liebold (29) modified spinning cup sequencer. Thus, problems in aligning overlapping fragments, such as those in Li and Chung's approach (7, 20), were avoided. Most of Li and Chung's most recent data (20) have been confirmed by this investigation, but careful analysis of their alignments raises doubts as to whether the discrepant segments of their proposed sequence are sufficiently documented.

It is interesting that the amino acid sequence of human βLPH is consistent with a nucleotide sequence (Fig. 3) containing only three bases in addition to those reported by Chang *et al.* (2). Insertion of adenine after nucleotide 773 would change the alanine-18 codon to GAC, specifying aspartic acid, and would also shift the reading frame so that the next five residues would agree with the sequence determined in this study. Insertions of cytosine after nucleotide 789 and any of the four bases after nucleotide 793 in the sequence proposed by Chang *et al.* (2) would generate the codons for glutamine-24 and alanine-25, respectively, and also return to the correct reading frame. The nucleotide sequence reported by Chang *et al.* (2) has two nearly adjacent 5′ C-C-$\frac{A}{T}$-G-G 3′ sequences (Fig. 3). The second cytosine in this sequence has been shown to be methylated in plasmid colicin E1 DNA extracted from *Escherichia coli* containing deoxycytosine methylase (36), such as *E. coli* strain

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                 5                  10                  15                  20                  25                  30
a  H-Glu Leu Thr Gly Gln Arg Leu Arg Glu Gly Asp Gly Pro Asp Gly Pro Ala Asp Asp Gly Ala Gly Ala Gln Ala Asp Leu Glu His Ser
     GAG CTG ACT GGG CAG CGA CTC CGG GAG GGA GAT GGC CCC GAC GGC CCC GCC GAC GAC GGC GCA GGG GC[C CAG G]CN GA[C CTG G]AG CAC AGC
                                                                      ↑                        ↑   ↑

                 5                  10                  15                  20                  25
b  H-Glu Leu Thr Gly Gln Arg Leu Arg Glu Gly Asp Gly Pro Asp Gly Pro Ala [Ala] [Thr] [Ala] [Gln] Gly [Pro] [Gly] [---] Asp Leu Glu His Ser
     GAG CTG ACT GGG CAG CGA CTC CGG GAG GGA GAT GGC CCC GAC GGC CCC GCC GCG ACG GCG CAG GGG [CCA GG]C --- GA[C CTG G]AG CAC AGC
             730             740             750             760             770             780          790               800

                 5                  10                  15                  20                  25                  30
c  H-Glu Leu Thr Gly Gln Arg Leu Arg [Gln] Gly Asp Gly Pro [Asn] [Ala] [Gly] Ala Asp Asp Gly [Pro] Gly Ala Gln Ala Asp Leu Glu His Ser
     GAG CTG ACT GGG CAG CGA CTC CGG CAG GGA GAT GGC CCC AAC GCC GGC GCC GAC GAC GGC CCA GGG GC[C CAG G]CN GA[C CTG G]AG CAC AGC
                                     *                   *   *   **      ↑           *             ↑   ↑

                 35                  40                  45↓                 50                  55                  60
d    Leu Leu Val Ala Ala Glu Lys Lys Asp Glu Gly Pro Tyr Arg Met Glu His Phe Arg Trp Gly Ser Pro Pro Lys Asp Lys Arg Tyr Gly

               ↓ 65                  70                  75                  80                  85
     Gly Phe Met Thr Ser Glu Lys Ser Gln Thr Pro Leu Val Thr Leu Phe Lys Asn Ala Ile Ile Lys Asn Ala Tyr Lys Lys Gly Glu-OH
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FIG. 3. Amino acid sequence of human βLPH. The sequence of the first 30 of the 46 residues obtained by direct sequence analysis of purified βLPH in this study (a) is compared with that predicted by Chang *et al.* (2) (b) on the basis of genomic DNA nucleotide sequence analysis and that proposed by Li and Chung (20) (c) on the basis of direct amino acid analysis. The nucleotide sequence shown under the amino acid sequence predicted by Chang *et al.* (2) (b) is that of the DNA fragment analyzed by those authors and is numbered accordingly. The same sequence, modified as necessary to code for the amino acid sequence reported in this study (a) and that of Li and Chung (20) (c), is shown under the corresponding amino acid sequence. From residue 31 on, only one sequence is shown (d) because there is no disagreement between the results of this study and the sequences proposed by Li and Chung (7, 20) and Cseh *et al.* (6) and predicted by Chang *et al.* (2). ↑, Bases inserted in the nucleotide sequence (2) to code for the amino acid sequence of βLPH determined in this study and proposed by Li and Chung (20); *, bases substituted in the nucleotide sequence to code for the structure proposed by Li and Chung (20); □, amino acid residues different from those found in this study; ⬚, 5′ C-C-$\frac{A}{T}$-G-G 3′ sequences; ↓, CNBr cleavage sites.

SR1592 used by Chang *et al.* (2). This cytosine does not appear in sequencing gels in the Maxam–Gilbert method (36, 37) but can be detected by locating the corresponding guanine band in the analysis of the complementary DNA strand (37). The inserted cytosine would be the second base in the first 5′ C-C-$^{A}_{T}$-G-G 3′ site in the DNA sequence reported by Chang *et al.* (20), and the third inserted nucleotide would lie between the two nearly adjacent sites (Fig. 3) in an area that might have presented difficulty during sequence analysis. An additional six base substitutions would be required to code for the revised amino acid sequence proposed by Li and Chung (20) (Fig. 3).

Since this manuscript was completed, the entire proOLMC gene from a single human placenta was characterized by Takahashi *et al.* (38), and the NH_2-terminal sequence of human βLPH, purified with a procedure different from the one used here, was determined by Hsi *et al.* (39). The sequence data of Takahashi *et al.* (38) and Hsi *et al.* (39) are in complete agreement with our results. Hsi *et al.* (39) did not provide data about the COOH-terminal structure of the purified peptide. However, in view of the lack of variation observed in this part of human βLPH (refs. 2, 6, 7, and 20; this work), it is probable that the NH_2-terminal sequence reported by Hsi *et al.* (39) belongs to the same peptide as that analyzed here.

Although the possibility that the discrepancies between the reported NH_2-terminal amino acid sequences of human βLPH reflect polymorphism within the proOLMC gene or gene multiplicity cannot be excluded, it seems more probable, on the basis of the recent investigations (refs. 38 and 39; this work) discussed above, that these deviations reflect minor analytical errors and that the results of Takahashi *et al.* (38), Hsi *et al.* (39), and this work establish the NH_2-terminal sequence of human βLPH.

Because human γLPH represents βLPH-(1–56)‡ (1), our sequence results and those of Hsi *et al.* (38) also establish the NH_2-terminal primary structure of human γLPH, which differs from that proposed by Li *et al.* (40).

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