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Complete Amino Acid Sequences of a Pair of Fish (Tilapia) Prolactins, tPRL₁₇₇ and tPRL₁₈₈*

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The complete amino acid sequences of a pair of tilapia (*Oreochromis mossambicus*) prolactins (PRLs) were determined. The larger PRL of molecular mass 20,836 Da consists of 188 amino acid residues. The smaller PRL of molecular mass 19,584 Da is 11 residues shorter. On alignment of the two sequences, the 19.6-kDa PRL (tPRL₁₇₇) has two conspicuous deletions on the NH₂-terminal side of the disulfide bond which connects the first and second cysteine residues. The degree of similarity between the two PRL sequences is unexpectedly low (130 identical residues, 69%) compared with that between the variants of other teleostean PRLs. Circular dichroism spectra and hydropathy profiles suggest structural similarity of the two PRLs. The sequence of the 20.8-kDa PRL (tPRL₁₈₈) has 69% identity with that of salmon PRL. The sequence of tPRL₁₇₇ is 56% identical with that of salmon PRL. Each tilapia PRL is equally similar to mammalian PRLs (about 30% identical residues). Regions highly conserved among teleostean and mammalian PRLs were identified on the COOH-terminal side of the disulfide bond connecting the first and second cysteine residues.

The pituitary hormone prolactin (PRL)¹ belongs to a family of molecules that are related structurally and functionally. The family includes pituitary growth hormone (GH), placental lactogen, and placental and cell line-derived proliferin (1-5). Within mammalian species, PRLs occur in multiple molecular forms, variously called variants, isohormones, or isoforms, which differ in primary structure and/or degree of glycosylation (6-9).

Among teleost fishes, two kinds of hormone variants have been reported. Specker *et al.* (10) reported the NH₂-terminal amino acid sequences of a pair of PRLs isolated from the tilapia (*Oreochromis mossambicus*). Within the first 26 residues there were five substitutions, suggesting moderate identity (81%). However, Yasuda *et al.* (11, 12) have reported the complete amino acid sequences of pairs of PRLs from the chum salmon (*Oncorhynchus keta*) and from the common carp

(*Cyprinus carpio*); these isohormones, in contrast with the pair of tilapia PRLs, are highly similar, differing from each other by only 4 residues and 1 residue, respectively. All fish PRLs lack 12 NH₂-terminal residues present in mammalian PRLs.

The pair of PRLs released in equal quantities from cultured tilapia pituitary glands are chemically distinct, with isoelectric points of 6.7 and 8.7 for the PRLs of mass 20 and 24 kDa, respectively, as estimated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, yet their immunogenic and physiologic properties are similar (10, 13). Both show identical activity in a bioassay designed to test for the osmoregulatory function of PRLs (14, 15); specifically, both PRLs prevented the loss of Na⁺ from hypophysectomized tilapia in fresh water (10). However, only the larger tilapia PRL promoted an increase in the length and weight of intact juvenile tilapia (13).

We now describe the determination of the complete amino acid sequences of these two tilapia PRLs. Comparison between them and other teleostean and mammalian PRLs may contribute toward understanding the relationships between structure and biological properties of PRLs and GHs.

EXPERIMENTAL PROCEDURES

Materials—Tilapia 20- and 24-kDa PRLs were purified as described by Specker *et al.* (10). The enzymes used for fragmentation were as follows: lysyl endopeptidase (Wako Pure Chemical), *Staphylococcus aureus* protease (Miles Laboratories Inc.), α -chymotrypsin (Sigma), and carboxypeptidase A (Sigma). Reagents and solvents for the gas-phase sequenator (ABI model 470A) were purchased from Applied Biosystems. Other chemicals used were of the purest grade commercially available.

Reduction and S-Carboxamidomethylation—Each tilapia PRL (100 μ g) was reduced with dithiothreitol (20 mM) in 100 μ l of 0.1 M Tris-HCl, 6 M guanidine HCl, 1 mM Na₂EDTA, pH 8.3, for 2 h at 50 °C and subsequently S-alkylated with iodoacetamide (45 mM) for 30 min at room temperature. S-Alkylated protein was desalted by reverse-phase high performance liquid chromatography (HPLC) on a YMC AM-312 ODS column (Yamamura Kagaku, 0.6 \times 15 cm, particle size 5 μ m, pore size 120 Å), eluted with a 60-min linear gradient of 0-70% aqueous acetonitrile containing 0.1% trifluoroacetic acid.

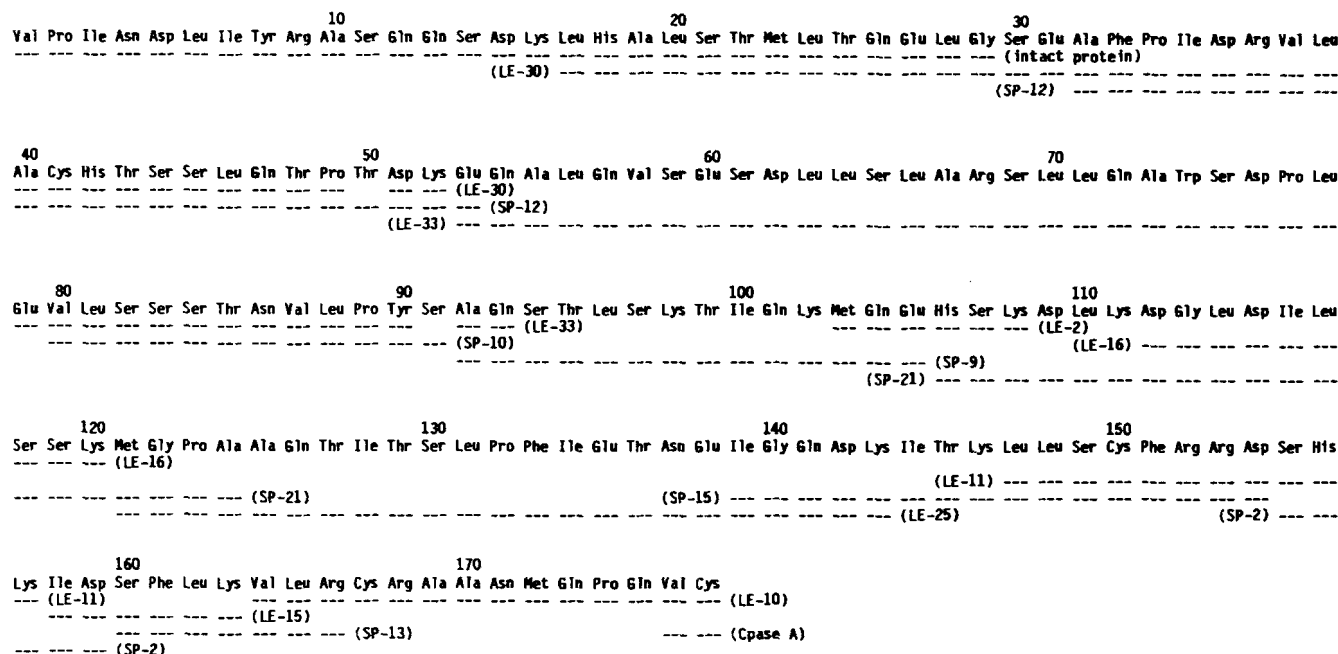
Enzymatic and Chemical Cleavage—S-Alkylated protein prepared as described above was subjected to enzymatic and chemical cleavage. For each cleavage, 2-4 nmol of protein were used. Digestion with lysyl endopeptidase was performed in 0.1 M Tris-HCl, 4 M urea, pH 9.0, at 37 °C for 4 h using 1% (w/w) enzyme. Digestion with *S. aureus* protease was performed in 0.1 M Tris-HCl, pH 7.8, at 37 °C for 24 h using 4% (w/w) enzyme. Digestion with chymotrypsin was performed in 0.1 M ammonium bicarbonate, 0.1 mM CaCl₂, pH 8.4, at 37 °C for 16 h using 1% (w/w) enzyme. Cyanogen bromide cleavage was performed with a 100 M excess of the reagent in 70% formic acid at room temperature in the dark for 24 h. The resultant peptides were separated by reverse-phase HPLC under the same conditions as described

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¹ The abbreviations used are: PRL, prolactin; GH, growth hormone; HPLC, high performance liquid chromatography.

A



B

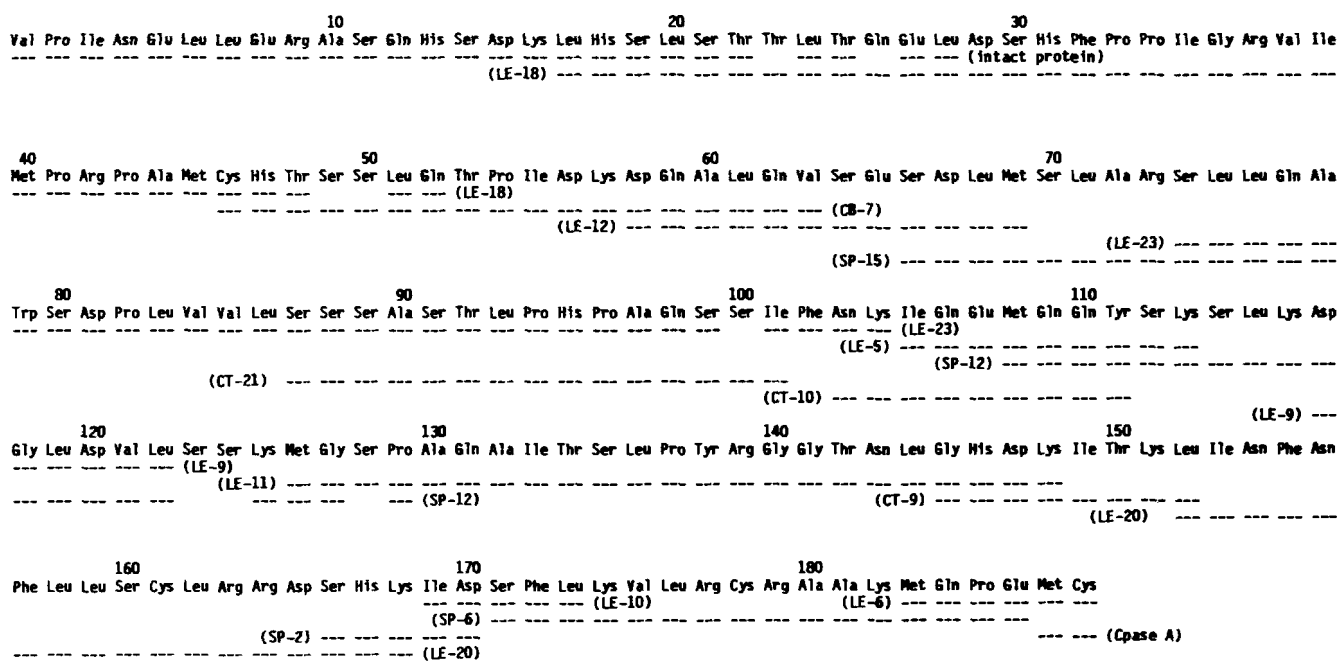


FIG. 1. A, complete amino acid sequence of 20-kDa tilapia PRL. Broken lines indicate amino acid residues determined by sequence analyses. The designations LE and SP represent peptides derived by lysyl endopeptidase and *S. aureus* protease, respectively. Cpase A represents the sequence derived by carboxypeptidase A digestion. B, complete amino acid sequence of the tilapia 24-kDa PRL. Broken lines indicate amino acid residues determined. The designations CT and CB represent peptides derived from chymotrypsin and from cyanogen bromide cleavages, respectively.

above. Carboxypeptidase digestion was performed in 0.2 M triethylamine formate, pH 8.5, at room temperature; aliquots were removed at appropriate time intervals and subjected to amino acid analysis.

Amino Acid Analysis—Two nmol of tilapia PRLs were hydrolyzed in 6 M HCl at 110 °C for 22 h. Amino acid analyses were carried out on a Jeol 200A amino acid analyzer with a standard gradient program. Amino acid analyses of the carboxypeptidase digests were performed by the Waters picoTag method of Bidlingmeyer *et al.* (16).

Sequence Analysis—Automated Edman degradation was performed

with a gas-liquid sequenator (17). Resultant phenylthiohydantoin were identified by HPLC on a C₈ reverse-phase column (Senshu Kagaku, SEQ-4, C₈, 0.46 × 30 cm, particle size 7 μm) at 40 °C using a gradient of acetonitrile in 40 mM sodium acetate buffer (pH 4.9) for elution.

Circular Dichroism Spectra—CD spectra were obtained on a Jasco 500A spectropolarimeter. Fused quartz cells of 5-mm path length were used for the measurements from 250 to 330 nm, and 0.2-mm path length for the measurements from 190 to 250 nm. Protein

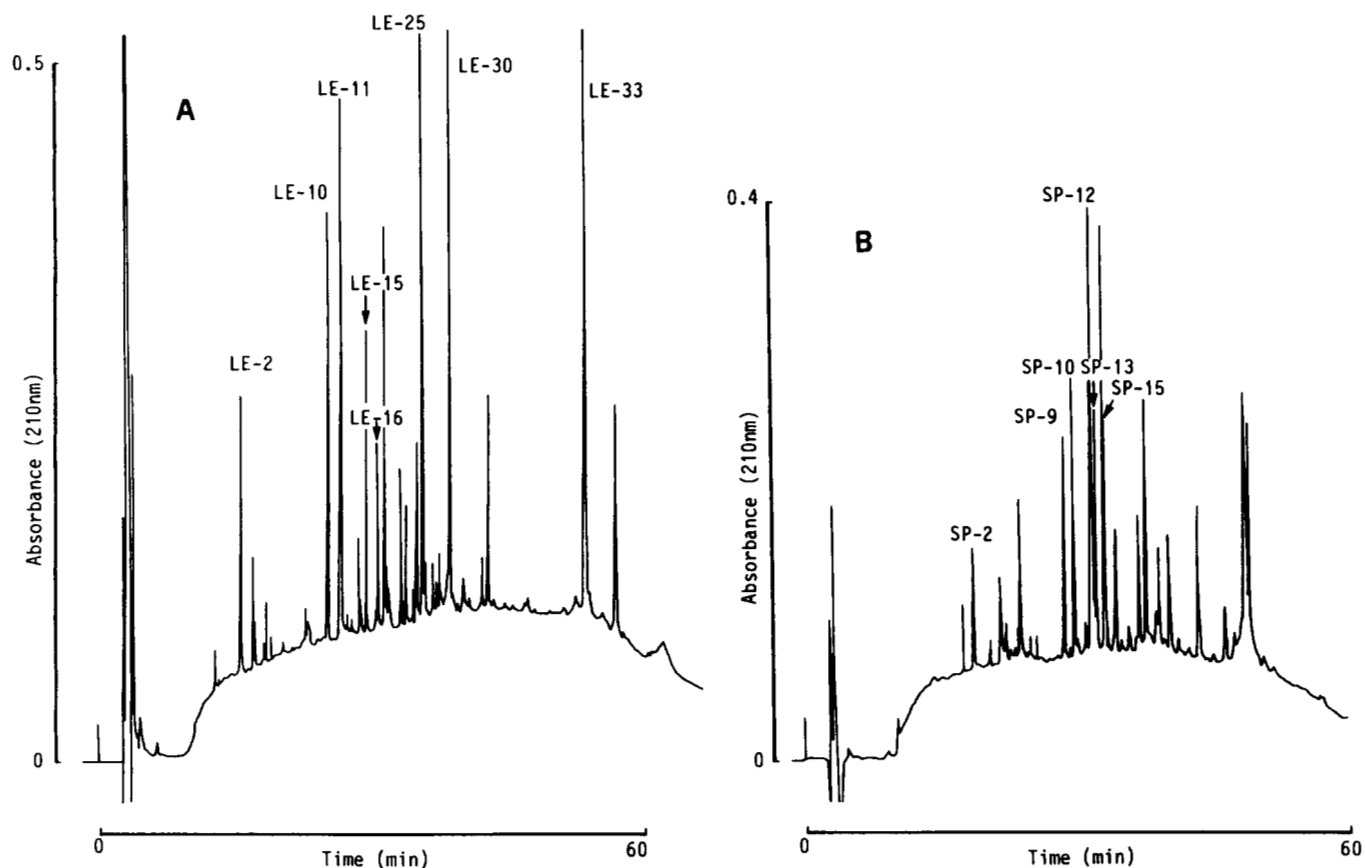


FIG. 2. A, separation by HPLC of a lysyl endopeptidase digest of 20-kDa PRL on a YMC AM-312 column (0.6 \times 15 cm, particle size 5 μ m). Elution was performed with a 60-min linear gradient of 0–70% acetonitrile containing 0.1% trifluoroacetic acid at a flow rate of 1 ml/min. B, separation by HPLC of a *S. aureus* digest of tilapia 20-kDa PRL. Chromatographic conditions are as in A.

concentrations were 0.2 mg/ml in water. Mean residue molecular ellipticities, $[\theta]_{MRW}$, were calculated using a value of 111 for the mean residue weight in both PRLs.

RESULTS

Amino Acid Sequences—Fig. 1 summarizes the amino acid sequences of the tilapia PRLs and the peptides used for sequence determination. Sequence data for the peptides are shown in Table 2.² Sequence analysis of 1 nmol of the intact *S*-carboxamidomethylated tilapia 20-kDa PRL allowed assignment of 29 NH_2 -terminal residues. In order to obtain the sequences of the remaining residues, further sequence analyses of fragments from proteolytic cleavage were performed. Eight lysyl endopeptidase peptides (LE-2, LE-10, LE-11, LE-15, LE-16, LE-25, LE-30, LE-33) purified by HPLC (Fig. 2A) were analyzed for NH_2 -terminal sequences. Peptide LE-30 confirmed a portion of the NH_2 -terminal sequence of the intact protein and extended it through residue 52. The other lysyl endopeptidase peptides provided the determination of a total of 109 additional residues. Subsequently, seven *S. aureus* protease peptides (SP-2, SP-9, SP-10, SP-12, SP-13, SP-15, SP-21) were isolated by HPLC (Fig. 2B). They provided 17 missing residues and the alignment of lysyl endopeptidase peptides in the order LE-30, LE-33, LE-2, LE-16, LE-25, LE-11, LE-15, and LE-10. Peptide SP-10 was found to be gener-

ated by nonselective cleavage with *S. aureus* protease at the carboxyl side of Ser-91. LE-30 and LE-33 overlapped by only one glutamyl residue. However, LE-33 was the only lysyl endopeptidase peptide having an NH_2 -terminal glutamic acid. In addition, the extensive sequence similarity between tilapia 20-kDa PRL and other teleostean PRLs (see below) confirmed the alignment.

Carboxypeptidase A digestion of the intact *S*-carboxamidomethyl protein provided the COOH-terminal sequence -Val-Cys (Table 3), and the complete sequence was accordingly established.

The strategy for sequence determination of 24-kDa PRL was similar to that used for 20-kDa PRL (Fig. 1 and Table 4). One nmol of the intact *S*-carboxamidomethylated 24-kDa PRL was submitted to sequence analysis, and an NH_2 -terminal sequence of 26 residues was determined. Analyses of nine lysyl endopeptidase peptides (LE-5, LE-6, LE-9, LE-10, LE-11, LE-12, LE-18, LE-20, LE-23 (Fig. 3A)) and four *S. aureus* protease peptides (SP-2, SP-6, SP-12, SP-15, Fig. 3B) provided the sequences of a total of 143 residues. Nine hitherto unidentified residues and two overlaps were established by analyses of peptides CT-9, CT-10, CT-21, and CB-7 obtained by chymotryptic digestion and cyanogen bromide cleavage (Fig. 3, C and D). CT-9 overlaps LE-20 by only 1 residue, Leu-151. However, LE-20 was the only lysyl endopeptidase peptide having an NH_2 -terminal leucine with the exception of LE-18, which had been aligned at 17–57. The considerable sequence similarity between tilapia 24-kDa PRL and tilapia 20-kDa PRL (see below) confirmed the alignment. Carboxypeptidase A digestion of intact *S*-carboxamidomethylated protein provided the COOH-terminal sequence -Met-Cys (Table

² Tables 2–5 are presented in miniprint at the end of this paper. Miniprint is easily read with the aid of a standard magnifying glass. Full size photocopies are included in the microfilm edition of the Journal that is available from Waverly Press.

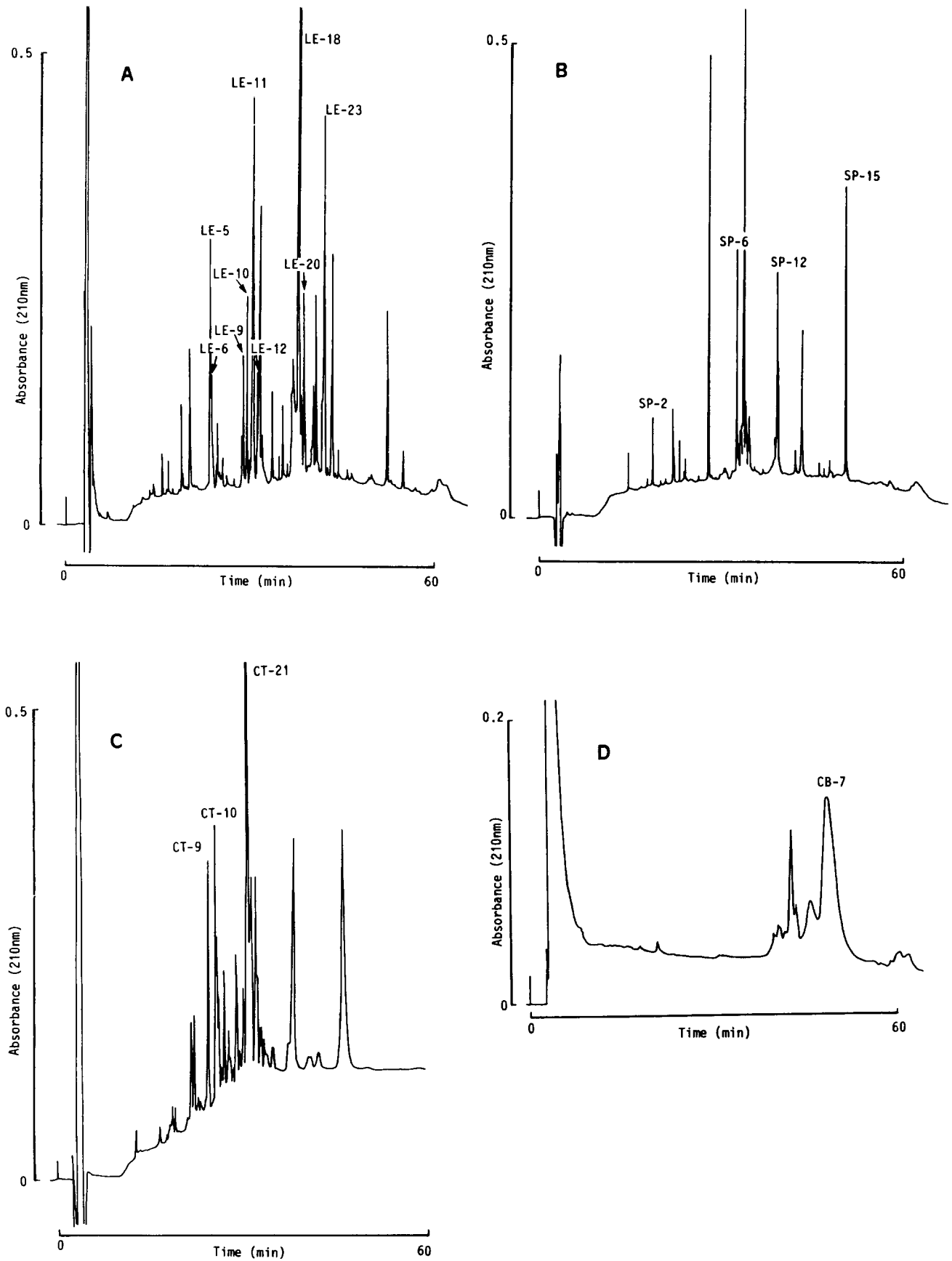


FIG. 3. A, separation by HPLC of a lysyl endopeptidase digest of tilapia 24-kDa PRL. Chromatographic conditions are as in Fig. 2A. B, separation by HPLC of a *S. aureus* protease digest of tilapia 24-kDa PRL. Chromatographic conditions are as in Fig. 2. C, separation by HPLC of a chymotrypsin digest of tilapia 24-kDa PRL. Chromatographic conditions are as in Fig. 2. D, separation by HPLC of the peptides generated by cyanogen bromide cleavage of 24-kDa PRL. Chromatographic conditions are as in Fig. 2.

5). Thus, the complete amino acid sequence of 24-kDa PRL was established.

The amino acid compositions of the two PRLs, shown in Table 1, are in agreement with the results of amino acid sequence determinations. Molecular masses were calculated to be 19,584 Da for 20-kDa PRL and 20,836 Da for 24-kDa PRL. The mass calculated for the 24-kDa PRL is lower than that estimated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (10). Sequence analyses confirmed that it has no glycosylation site such as is present in mammalian PRLs (7).

CD Spectra—The CD spectra of the two PRLs are shown in Fig. 4. The two spectra are similar in the region of amide bond absorption (Fig. 4A). The position of two negative bands

at 209 nm and 221 nm in this region is typical of α -helical polypeptides. The relative intensities of these two bands are similar to those of human GH reported by Bewley *et al.* (18). The α -helix content estimated by the method of Bewley and Li (19) is about 45% in each of the PRLs. The CD spectrum

TABLE I
Amino acid composition of 20- and 24-kDa PRL

Residue	20 kDa	24 kDa
Asx	16.9 ^a (16) ^b	16.9 (16)
Thr	11.9 (12)	10.2 (9)
Ser	20.8 (24)	23.1 (27)
Glx	21.3 (22)	18.6 (18)
Pro	7.9 (8)	12.2 (12)
Gly	5.0 (4)	6.9 (6)
Ala	13.1 (12)	11.7 (11)
½Cys	3.5 (4)	3.6 (4)
Val	6.7 (7)	6.0 (7)
Met	3.7 (4)	6.6 (7)
Ile	9.8 (10)	9.9 (10)
Leu	26.6 (25)	27.1 (26)
Tyr	1.1 (2)	1.6 (2)
Phe	4.5 (4)	4.9 (5)
His	4.7 (4)	7.3 (7)
Lys	11.6 (11)	11.4 (11)
Arg	7.2 (7)	9.1 (9)
Trp	ND ^c (1)	ND (1)
Total	176.3 (177)	187.1 (188)

^a Values indicate the number of residues/molecule.
^b Numbers in parentheses represent the number of residues determined by sequence analysis.
^c ND, not determined.

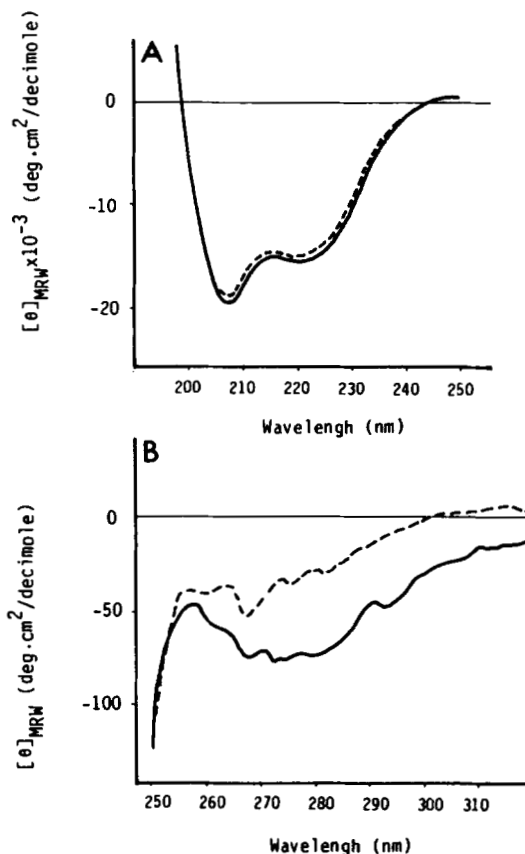


FIG. 4. Circular dichroism spectra of 20-kDa PRL (—) and the 24-kDa PRL (---) in H₂O. A, amide bond circular dichroism spectra. B, side-chain circular dichroism spectra.

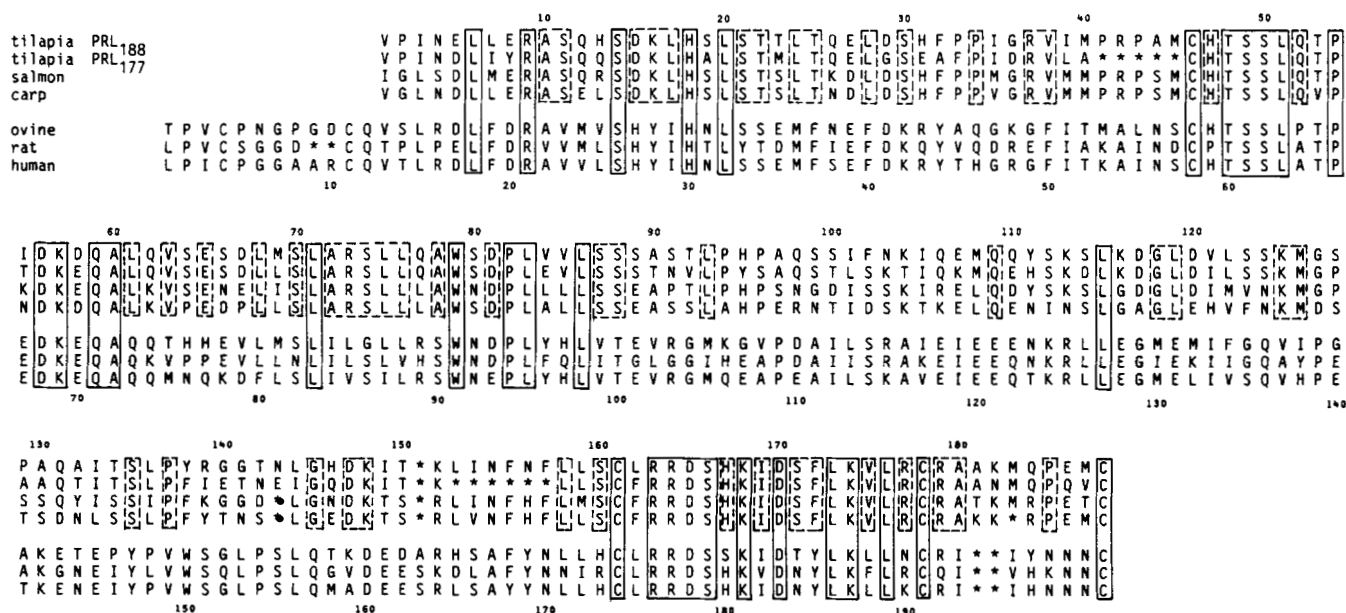


FIG. 5. Alignment of amino acid sequences (represented by standard single-letter abbreviations) of tPRL₁₇₇ and tPRL₁₈₈ with those of PRLs from salmon (11), carp (12), sheep (20), rat (21), and human (22). Identical residues among the teleost PRLs are boxed with a broken line. Identical residues in teleost and mammal PRLs are boxed with a solid line.

in the region of aromatic side chain absorption shows some differences; neither spectrum shows a positive tryptophan band above 290 nm, which was observed in ovine PRL and human GH (18). At the present time we have no interpretation to offer for these findings.

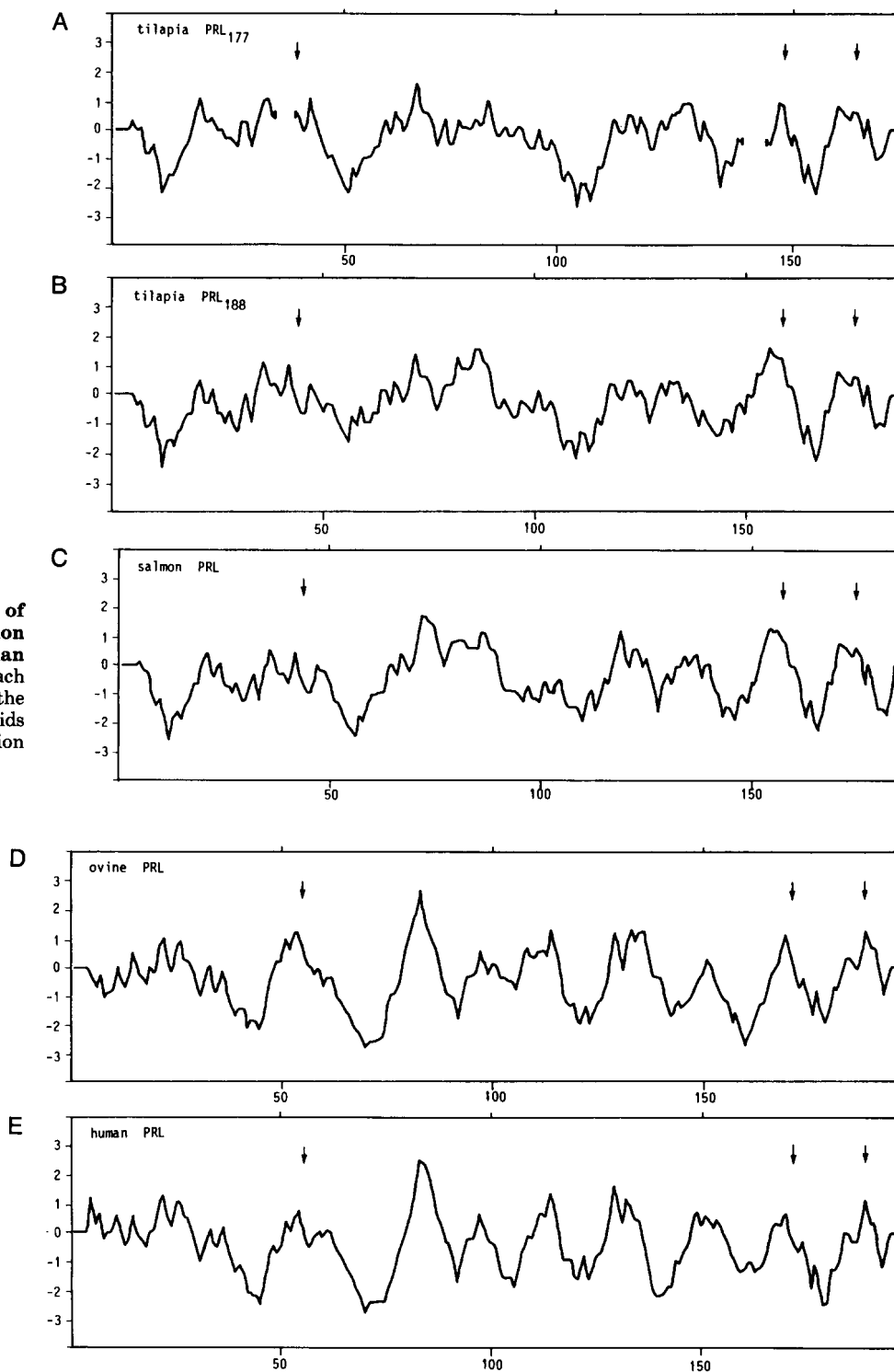
DISCUSSION

The complete amino acid sequences of the two tilapia PRLs surprisingly disclose much less similarity than exists between the two salmon PRLs and between the two carp PRLs. The sequence identity for the entire molecule is only 69% (130/188), considerably less than that found in the NH₂-terminal

sequence (81%, 21/26) (10). The larger tilapia PRL, previously termed 24-kDa PRL and henceforth termed tPRL₁₈₈, contains 188 residues. The smaller tilapia PRL, previously termed 20-kDa PRL and henceforth called tPRL₁₇₇, contains 177 residues. The more basic isoelectric point of tPRL₁₈₈ (8.7) compared with that of tPRL₁₇₇ (6.7) (10) can now be explained by the larger number of basic residues and the smaller number of acidic residues.

Fig. 5 details the complete amino acid sequences of the two tilapia PRLs, along with those of some other teleostean and mammalian PRLs. Sequences have been aligned to optimize similarity. The teleostean PRLs all lack the first 12 amino

FIG. 6. Hydropathy profiles of tPRL₁₇₇ (A), tPRL₁₈₈ (B), salmon PRL (C), ovine PRL (D), and human PRL (E). A hydropathy score for each residue was obtained by averaging the hydropathy indices (34) of 9 amino acids at a time. Arrows indicate the position of cysteine residues.



acid residues present in mammalian PRLs and, consequently, the first disulfide loop that exists in mammalian PRLs. In having similar numbers of residues and four conserved cysteine residues rather than six, they more closely resemble GHs. Each teleostean PRL is only about 30% identical to the mammalian PRLs. tPRL₁₇₇ is about 56% (105/188) identical to salmon PRL and 51% (96/188) identical to carp PRL. tPRL₁₈₈ is 69% (129/188) identical to salmon PRL and 64% (121/188) identical to carp PRL.

The degree of identity between the two tilapia PRLs (69%) is no higher than that between tilapia PRLs and the other teleostean PRLs. Compared to tPRL₁₈₈, tPRL₁₇₇ lacks two short sequences: 5 residues (41–45) and 6 residues (152–157) preceding the first cysteines of the two disulfide bonds which are likely to be present (see below). The 58 substitutions found between the two tilapia PRLs are distributed along the entire molecule. This extends our earlier contention that these two forms are distinct at the pretranslational level (10, 13) and are probably products of two separate genes. Further, the greater similarity of the larger than the smaller tilapia PRL to the other teleostean PRLs (69 and 64% compared with 56 and 51%) suggests that receptor systems and bioassay systems might distinguish tPRL₁₇₇ from other teleostean PRLs. In this regard, it is interesting that in intact juvenile tilapia, ovine PRL, bovine GH, and the larger tilapia PRL were found to stimulate growth, whereas neither tilapia GH nor the smaller tilapia PRL had such an effect (13).

In an amphibian bioassay system, tPRL₁₇₇, but not tPRL₁₈₈ nor various GHs, behaves similarly to ovine and amphibian PRL.³ We might speculate that overall these findings suggest that conservative evolutionary pressure on the tilapia PRLs remained on the larger, leaving the smaller one free to change and take on other functions or to lose original functions. Specker *et al.* (23) proposed that the PRL cell is a renegade growth hormone cell. We can now propose that tPRL₁₇₇ is a renegade PRL molecule which has lost growth hormone-like characteristics.

Identical residues among the teleostean and the mammalian PRLs (see Fig. 5) are clustered in four highly conserved regions located at the alignment positions 6–20, 46–60, 71–86, and 161–178. Residues involved in receptor binding are thought to be located within these highly conserved regions, since ovine PRL shows high binding affinity to the tilapia PRL receptor (24), and conversely, PRLs of some teleosts have low but significant activity in mammalian PRL assays (25–27). Recently, Nicoll *et al.* (4) compared the amino acid sequences of mammalian GHs and PRLs in an attempt to identify the regions involved in receptor binding and in ensuring hormone specificity and generating species specificity. They suggested that four clusters of residues are the determinants for receptor binding corresponding to positions 3–8, 50–57, 78–82, and 127–136 of the teleostean PRL sequences. Among these, only the first three clusters correspond with regions highly conserved between teleostean and mammalian PRL sequences and are thus likely to contain determinants for receptor binding of teleostean PRL.

Because the 1–134 segment of human GH has binding affinity for both mammalian PRL and GH receptors (28, 29), the NH₂-terminal two-thirds of PRL and GH are thought to be important for receptor binding. However, the 1–134 fragment has low potency *in vivo* (30). In contrast, two-chain forms of human GH obtained by enzymatic cleavage in the region between residues 134 and 150 (31, 32) and two-chain forms of rat PRL cleaved at about the same position (33) retain full activity in *in vivo* mammalian assays. These results

suggest that COOH-terminal portions of the molecules ensure hormone specific activity *in vivo*. Teleostean and mammalian PRLs are thought to have similar tertiary structures as judged from sequence similarity, similar circular dichroism spectra in the region of amide bond absorption, and similar hydrophathy profiles (Fig. 6). Therefore, teleostean PRLs probably have two disulfide bonds formed by cysteines 46–161 and cysteines 178–188, corresponding to the similarly located disulfide bonds present in mammalian PRLs (20). The closest similarity between the PRLs is on the COOH-terminal side of cysteine 161; another highly similar region is on the COOH-terminal side of cysteine 46. These highly conserved regions are thought to be exposed to the outside of the molecule as indicated by low hydrophathy values (Fig. 6) and are probably essential for biological activity of mammalian PRLs as well as teleostean PRLs.

The new information on the structures of this pair of PRLs supports our earlier conclusion, based more on biological and immunological information (10, 13), that the tilapia PRLs are importantly similar, and possibly importantly different, in their tertiary structures. Thus, one conclusion, possibly important to efforts to modify PRL or GH functions using recombinant DNA techniques, is that two deletions from tPRL₁₇₇, both located on the NH₂-terminal side of disulfide bonds, are of no consequence to the tertiary structure which ensures PRL's osmoregulatory function; however, their loss, and/or alteration in residues, seems to have led to decreased effectiveness in promoting juvenile growth.

REFERENCES

1. Miller, W. L., and Eberhardt, N. L. (1983) *Endocr. Rev.* **4**, 97–130
2. Wallis, M. (1981) *J. Mol. Evol.* **17**, 10–18
3. Niall, H. D., Hogan, M. L., Saver, R., Rosenblum, I. Y., and Greenwood, F. C. (1971) *Proc. Natl. Acad. Sci. U. S. A.* **68**, 866–869
4. Nicoll, C. S., Mayer, G. L., and Russell, S. M. (1986) *Endocr. Rev.* **7**, 169–203
5. Linzer, D. I. H., Lee, S.-J., Ogren, L., Talamantes, F., and Nathans, D. (1985) *Proc. Natl. Acad. Sci. U. S. A.* **82**, 4356–4359
6. Meuris, S., Svoboda, M., Vilamala, M., Christophe, J., and Robyn, C. (1983) *FEBS Lett.* **154**, 111–115
7. Lewis, U. J., Singh, R. N. P., Lewis, L. J., Seavey, B. K., and Sinha, Y. N. (1984) *Proc. Natl. Acad. Sci. U. S. A.* **81**, 4255–4259
8. Pansini, F., Bergamini, C. M., Malfaccini, M., Cocilovo, G., Linciano, M., Jacobs, M., and Bagni, B. (1985) *J. Endocrinol.* **106**, 81–85
9. Oetting, W. S., and Walker, A. M. (1986) *Endocrinology* **119**, 1377–1381
10. Specker, J. L., King, D. S., Nishioka, R. S., Shirahata, K., Yamaguchi, K., and Bern, H. A. (1985) *Proc. Natl. Acad. Sci. U. S. A.* **82**, 7490–7494
11. Yasuda, A., Itoh, H., and Kawauchi, H. (1986) *Arch. Biochem. Biophys.* **244**, 528–541
12. Yasuda, A., Miyazima, K., Kawauchi, H., Peter, R. E., Lin, H.-R., Yamaguchi, K., and Sano, H. (1987) *Gen. Comp. Endocrinol.* **66**, 280–290
13. Specker, J. L., King, D. S., Rivas, R. J., and Young, B. K. (1985) in *Prolactin-Brain and Clinical Correlates* (MacLeod, R. M., Thorner, M. O., and Scapagnini, U., eds) Vol. 1, pp. 427–436, Fidia Research Series, Liviana Press, Padova, Italy
14. Clarke, W. C., and Bern, H. A. (1980) in *Hormonal Proteins and Peptides* (Li, C. H., ed) Vol. 8, pp. 105–197, Academic Press, Orlando, FL
15. Loretz, C. A., and Bern, H. A. (1982) *Neuroendocrinology* **35**, 292–304
16. Bidlingmeyer, B. A., Cohen, S. A., and Tarvin, T. L. (1984) *J. Chromatogr.* **336**, 93–104
17. Hewick, R. M., Hunkapiller, M. W., Hood, L. E., and Dreyer, W. J. (1981) *J. Biol. Chem.* **256**, 7990–7997
18. Bewley, T. A., Brovetto-Cruz, J., and Li, C. H. (1969) *Biochemistry* **8**, 4701–4708

³ J. L. Specker, S. C. Brown, and P. S. Brown, unpublished data.

19. Bewley, T. A., and Li, C. H. (1972) *Biochemistry* **11**, 884-888
20. Li, C. H. (1976) *Int. J. Pept. Protein Res.* **8**, 205-224
21. Gubbins, E. J., Maurer, R. A., Lagrimini, M., Erwin, C. R., and Donelson, J. E. (1980) *J. Biol. Chem.* **255**, 8655-8662
22. Cooke, N. E., Coit, D., Shine, J., Baxter, J. D., and Martial, J. A. (1981) *J. Biol. Chem.* **256**, 4007-4016
23. Specker, J. L., Ingleton, P. M., and Bern, H. A. (1984) in *Prolactin Secretion: A Multidisciplinary Approach* (Mena, F., and Valverde-R., C. M., eds) pp. 17-30, Academic Press, Orlando, FL
24. Edery, M., Young, G., Bern, H. A., and Steiny, S. (1984) *Gen. Comp. Endocrinol.* **56**, 19-23
25. Houdebine, L. M., Farmer, S. W., and Prunet, P. (1981) *Gen. Comp. Endocrinol.* **45**, 61-65
26. Prunet, P., Djiane, J., and Breton, B. (1977) *J. Endocrinol.* **73**, 391-392
27. Prunet, P., and Houdebine, L. M. (1984) *Gen. Comp. Endocrinol.* **53**, 49-57
28. Reagan, C. R., Kostyo, J. L., Mills, J. B., and Wilhelmi, A. E. (1973) *Fed. Proc.* **32**, 265
29. Aston, R., and Ivanyi, J. (1983) *EMBO J.* **2**, 493-497
30. Li, C. H., and Graf, L. (1974) *Proc. Natl. Acad. Sci. U. S. A.* **71**, 1197-1201
31. Singh, R. N. P., Seavy, B. K., Rice, V. P., Lindsey, T. T., and Lewis, U. J. (1974) *Endocrinology* **94**, 883-891
32. Lewis, U. J., Singh, R. N. P., VanderLaan, W. P., and Tutwiler, G. F. (1977) *Endocrinology* **101**, 1587-1603
33. Mittra, I. (1980) *Biochem. Biophys. Res. Commun.* **95**, 1750-1759
34. Kyte, J., and Doolittle, R. F. (1982) *J. Mol. Biol.* **157**, 105-132

Supplementary Material to
Complete Amino Acid Sequences of a Pair of Fish(Tilapia)
Prolactins.

by
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Table 2. Amino acid sequence data for carboxamidomethylated
[PRL177]

Position	Residue	Peptide, (cycle), yield, (pmole)
		<u>Intact Protein</u>
1	Val	(1) 770
2	Pro	(2) 847
3	Ile	(3) 834
4	Asn	(4) 704
5	Asp	(5) 500
6	Leu	(6) 910
7	Ile	(7) 921
8	Tyr	(8) 728
9	Arg	(9) 668
10	Ala	(10) 860
11	Ser	(11) 150
12	Gln	(12) 510
13	Gln	(13) 675
14	Ser	(14) 120
15	Asp	(15) 175
16	Lys	(16) 460
17	Leu	(17) 532 (1) 1035
18	His	(18) 175 (2) 182
19	Ala	(19) 326 (3) 556
20	Leu	(20) 480 (4) 752
21	Ser	(21) 112 (5) 270
22	Thr	(22) 52 (6) 88
23	Met	(23) 195 (7) 440
24	Leu	(24) 440 (8) 554
25	Thr	(25) 66 (9) 86
26	Gln	(26) 198 (10) 300
27	Glu	(27) 120 (11) 182
28	Leu	(28) 310 (12) 350
29	Gly	(29) 86 (13) 200
30	Ser	(14) 68 <u>SP-12</u>
31	Glu	(15) 122
32	Ala	(16) 54 (1) 1075
33	Phe	(17) 178 (2) 905
34	Pro	(18) 102 (3) 674
35	Ile	(19) 146 (4) 830
36	Asp	(20) 70 (5) 270
37	Arg	(21) 48 (6) 135
38	Val	(22) 113 (7) 657
39	Leu	(23) 102 (8) 841
40	Ala	(24) 101 (9) 465
41	Cys	(25) 25 (10) 300
42	His	(26) 15 (11) 75
43	Thr	(27) 10 (12) 22
44	Ser	(28) 42 (13) 83
45	Ser	(29) 46 (14) 125
46	Leu	(30) 41 (15) 132
47	Gln	(31) 14 (16) 86
48	Thr	(32) 5 (17) 15
49	Pro	(33) 13 (18) 36
50	Thr	(34) - (19) 16
51	Asp	(35) 5 (20) 12
52	Lys	(36) 10 (21) 15
53	Glu	(1) 434 (22) 4
54	Gln	(2) 888
55	Ala	(3) 863
56	Leu	(4) 905
57	Gln	(5) 717
58	Val	(6) 715
59	Ser	(7) 210
60	Glu	(8) 155
61	Ser	(9) 256
62	Asp	(10) 160
63	Leu	(11) 372
64	Leu	(12) 412
65	Ser	(13) 160
66	Leu	(14) 291
67	Ala	(15) 210
68	Arg	(16) 45
69	Ser	(17) 176
70	Leu	(18) 215
71	Leu	(19) 192
72	Gln	(20) 165
73	Ala	(21) 142
74	Trp	(22) 65
75	Ser	(23) 105
76	Asp	(24) 36
77	Pro	(25) 54

78	Leu	(26) 102	<u>SP-10</u>
79	Glu	(27) 28	
80	Val	(28) 72	(1) 636
81	Leu	(29) 100	(2) 539
82	Ser	(30) 72	(3) 240
83	Ser	(31) 85	(4) 240
84	Ser	(32) 76	(5) 202
85	Thr	(33) 6	(6) 43
86	Asn	(34) 18	(7) 211
87	Val	(35) 30	(8) 270
88	Leu	(36) 74	(9) 221
89	Pro	(37) 22	(10) 145
90	Tyr	(38) 24	(11) 192
91	Ser	(39) -	(12) 35
92	Ala	(30) 22	(1) 635
93	Gln	(41) 27	(2) 450
94	Ser		(3) 265
95	Thr		(4) 66
96	Leu		(5) 150
97	Ser		(6) 126
98	Lys		(7) 63
99	Thr		(8) 25
100	Ile		(9) 265
101	Gln	<u>LE-2</u>	(10) 178
102	Lys		(11) 60
103	Met	(1) 845	(12) 118
104	Gln	(2) 860	(13) 56
105	Glu	(3) 685	<u>SP-21</u>
106	His	(4) 103	(1) 102
107	Ser	(5) 454	(2) 40
108	Lys	(6) 295	(3) 65
109	Asp		(4) 46
110	Leu	<u>LE-16</u>	(5) 120
111	Lys		(6) 52
112	Asp	(1) 160	(7) 39
113	Gly	(2) 164	(8) 58
114	Leu	(3) 215	(9) 90
115	Asp	(4) 61	(10) 28
116	Ile	(5) 200	(11) 50
117	Leu	(6) 190	(12) 50
118	Ser	(7) 72	(13) 20
119	Ser	(8) 70	(14) 20
120	Lys	(9) 24	(15) 10
121	Met		(16) 14 (1) 522
122	Gly		(17) 19 (2) 392
123	Pro		(18) 8 (3) 445
124	Ala		(19) 16 (4) 680
125	Ala		(5) 590
126	Gln		(6) 488
127	Thr		(7) 118
128	Ile		(8) 536
129	Thr		(9) 78
130	Ser		(10) 140
131	Leu		(11) 305
132	Pro		(12) 180
133	Phe		(13) 230
134	Ile		(14) 85
135	Glu		(15) 36
136	Thr		(16) 30
137	Asn		(17) 74
138	Glu	<u>SP-15</u>	(18) 50
139	Ile	(1) 288	(19) 152
140	Gly	(2) 279	(20) 102
141	Gln	(3) 292	(21) 84
142	Asp	(4) 72	(22) 30
143	Lys	(5) 120	(23) 8
144	Ile	(6) 200	
145	Thr	(7) 54	<u>LE-11</u>
146	Lys	(8) 106	
147	Leu	(9) 228	(1) 1042
148	Leu	(10) 222	(2) 912
149	Ser	(11) 68	(3) 292
150	Cys	(12) 46	(4) 538
151	Phe	(13) 132	(5) 655
152	Arg	(14) 24	(6) 81
153	Arg	(15) 20	(7) 105
154	Asp	(16) 6	(8) 122
155	Ser		(9) 168 (1) 132
156	His		(10) 30 (2) 98
157	Lys	<u>LE-15</u>	(11) 32 (3) 145
158	Ile	(1) 1100	<u>SP-13</u>
159	Asp	(2) 624	(4) 210
160	Ser	(3) 294	(5) 36
161	Phe	(4) 745	
162	Leu	(5) 672	(1) 80
163	Lys	(6) 304	(2) 370
164	Val		(3) 384
165	Leu		(4) 300
166	Arg		(5) 352 (1) 1108
167	Cys		(6) 365 (2) 1080
168	Arg		(7) 392 (3) 116
169	Ala		(8) 80 (4) 442
170	Asn		(5) 106 (6) 434
171	Met		(7) 392 (8) 208
172	Gln		(9) 181
173	Gln		(10) 145
174	Pro		(11) 142
175	Gln		(12) 172
176	Val		(13) 43
177	Cys		(14) 8

Table 3. Carboxypeptidase A digestion of carboxamidomethylated tPR1188

Time (min)	Residues/mole	
	Val(176)	Carboxamidomethyl-cysteine(177)
10	0.21	0.37
30	0.82	0.87
120	0.92	1.0

Table 4. Amino acid sequence data for carboxamidomethylated tPR1188

Position	Residue	Peptide, (cycle), yield, (pmole)
<u>Intact Protein</u>		
1	Val	(1) 771
2	Pro	(2) 383
3	Ile	(3) 691
4	Asn	(4) 435
5	Glu	(5) 373
6	Leu	(6) 418
7	Leu	(7) 622
8	Glu	(8) 298
9	Arg	(9) 52
10	Ala	(10) 383
11	Ser	(11) 72
12	Gln	(12) 243
13	His	(13) 43
14	Ser	(14) 62
15	Asp	(15) 73
16	Lys	(16) 197
17	Leu	(17) 188
18	His	(18) 20
19	Ser	(19) 42
20	Leu	(20) 166
21	Ser	(21) 33
22	Thr	(22) 23
23	Thr	(23) -
24	Leu	(24) 115
25	Thr	(25) 10
26	Gln	(26) -
27	Glu	(27) 60
28	Leu	(28) 110
29	Asp	(29) 143
30	Ser	(30) 58
31	His	(31) 40
32	Phe	(32) 107
33	Pro	(33) 110
34	Pro	(34) 170
35	Ile	(35) 313
36	Gly	(36) 77
37	Arg	(37) 41
38	Val	(38) 87
39	Ile	(39) 112
40	Met	(40) 60
41	Pro	(41) 28
42	Arg	(42) 28
43	Pro	(43) 90
44	Ala	(44) 30
45	Met	(45) 25
46	Cys	(46) 17
47	His	(47) 9
48	Thr	(48) 7
49	Ser	(49) -
50	Ser	(50) -
51	Leu	(51) 14
52	Gln	(52) 11
53	Thr	(53) 16
54	Pro	(54) 76
55	Ile	(55) 71
56	Asp	(56) 80
57	Lys	(57) 59
58	Asp	(58) 36
59	Gln	(59) 56
60	Ala	(60) 45
61	Leu	(61) 66
62	Gln	(62) 33
63	Val	(63) 44
64	Ser	(64) 15
65	Glu	(65) 23
66	Ser	(66) 16
67	Asp	(67) 18
68	Leu	(68) 24
69	Met	(69) 6
70	Ser	(70) 35
71	Leu	(71) 79
72	Ala	(72) 86
73	Arg	(73) 22
74	Ser	(74) 186
75	Leu	(75) 431
76	Leu	(76) 430
77	Gln	(77) 365
78	Ala	(78) 258
79	T p	(79) 58
80	Ser	(80) 63
81	Asp	(81) 202
82	Pro	(82) 175
83	Leu	(83) 160
84	Val	(84) 128
85	Val	(85) 160
86	Leu	(86) 105
87	Ser	(87) 56
88	Ser	(88) 58
89	Ser	(89) 47
90	Ala	(90) 51

91	Ser	(91) 25	(5) 117
92	Thr	(92) 10	(6) 38
93	Leu	(93) 26	(7) 218
94	Pro	(94) 35	(8) 216
95	His	(95) 9	(9) 114
96	Pro	(96) 24	(10) 178
97	Ala	(97) 11	(11) 145
98	Gln	(98) 16	(12) 198
99	Ser	(99) 13	(13) 70
100	Ser	(100) -	(14) 41
101	Ile	(101) 11	(15) 10
102	Phe	(102) 11	
103	Asn	(103) 6	LE-5 (1) 762
104	Lys	(104) 6	(2) 556
105	Ile	(105) -	(3) 671
106	Gln	(106) -	(4) 578
107	Glu	(107) -	(5) 560
108	Met	(108) 60	(6) 630
109	Gln	(109) 62	(7) 381
110	Gln	(110) 54	(8) 380
111	Tyr	(111) 34	(9) 179
112	Ser	(112) 20	(8) 91
113	Lys	(113) 47	(9) 58
114	Ser	(114) 19	
115	Leu	(115) 45	LE-9 (1) 410
116	Lys	(116) 52	(2) 420
117	Asp	(117) 24	(3) 520
118	Gly	(118) 18	(4) 388
119	Leu	(119) 40	(5) 385
120	Asp	(120) 41	(6) 447
121	Val	(121) 33	(7) 177
122	Leu	(122) 28	(8) 186
123	Ser	(123) -	(9) 40
124	Ser	(124) -	(7) 45
125	Lys	(125) 16	LE-17 (1) 521
126	Met	(126) 18	(2) 573
127	Gly	(127) 19	(3) 102
128	Ser	(128) 21	(4) 467
129	Pro	(129) -	(5) 504
130	Ala	(130) 12	(6) 404
131	Gln	(131) -	(7) 401
132	Ala	(132) -	(8) 266
133	Ile	(133) -	(9) 125
134	Thr	(134) -	(10) 28
135	Ser	(135) -	(11) 168
136	Leu	(136) -	(12) 198
137	Pro	(137) -	(13) 215
138	Tyr	(138) -	(14) 71
139	Arg	(139) -	(15) 189
140	Gly	(140) -	(16) 198
141	Gly	(141) -	(17) 47
142	Thr	(142) -	(18) 114
143	Asn	(143) -	(19) 136
144	Leu	(144) -	(20) 102
145	Gly	(145) 580	(21) 26
146	His	(146) 298	(22) 98
147	Asp	(147) 559	(23) 16
148	Lys	(148) 677	
149	Ile	(149) 501	
150	Thr	(150) 26	LE-20 (1) 122
151	Lys	(151) 474	(2) 97
152	Leu	(152) 162	(3) 156
153	Ile	(153) -	(4) 119
154	Asn	(154) -	(5) 110
155	Phe	(155) -	(6) 90
156	Asn	(156) -	(7) 108
157	Phe	(157) -	(8) 135
158	Leu	(158) -	(9) 40
159	Ser	(159) -	(10) 58
160	Cys	(160) -	(11) 51
161	Leu	(161) -	(12) 44
162	Arg	(162) -	(13) 52
163	Arg	(163) -	(14) 105
164	Arg	(164) -	(15) 22
165	Asp	(165) -	(16) 30
166	Ser	(166) -	(17) 11
167	His	(167) -	(3) 160
168	Lys	(168) -	(4) 241
169	Ile	(169) 263	(5) 60
170	Asp	(170) 275	
171	Ser	(171) 45	
172	Phe	(172) 128	
173	Leu	(173) 100	
174	Lys	(174) 37	
175	Val	(175) -	
176	Leu	(176) -	
177	Arg	(177) -	
178	Cys	(178) -	
179	Arg	(179) -	
180	Ala	(180) -	
181	Ala	(181) -	
182	Lys	(182) -	
183	Met	(183) 1002	
184	Gln	(184) 843	
185	Pro	(185) 451	
186	Glu	(186) 625	
187	Met	(187) 319	
188	Cys	(188) 166	

Table 5. Carboxypeptidase A digestion of carboxamidomethylated tPR1188

Time (min)	Residues/mole	
	Met(187)	Carboxamidomethyl-cysteine(188)
10	-	0.09
30	0.07	0.24
120	0.15	0.71

CT-10
CT-17
CT-9
LE-18
CB-7
LE-12
SP-15
LE-23
CT-21

LE-5
LE-9
LE-17
CT-9
LE-10
SP-6
SP-2
LE-6