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Complete Amino Acid Sequences of a Pair of Fish (Tilapia) Prolactins, $tPRL_{177}$ and $tPRL_{188}$*

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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 ($tPRL_{177}$) has two conspicuous deletions on the NH_2-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 ($tPRL_{188}$) has 69% identity with that of salmon PRL. The sequence of $tPRL_{177}$ 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 NH_2-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 NH_2-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 μg) was reduced with dithiothreitol (20 mM) in 100 μl of 0.1 M Tris-HCl, 6 M guanidine HCl, 1 mM Na_2EDTA, 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 × 15 cm, particle size 5 μm, 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 $CaCl_2$, 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.

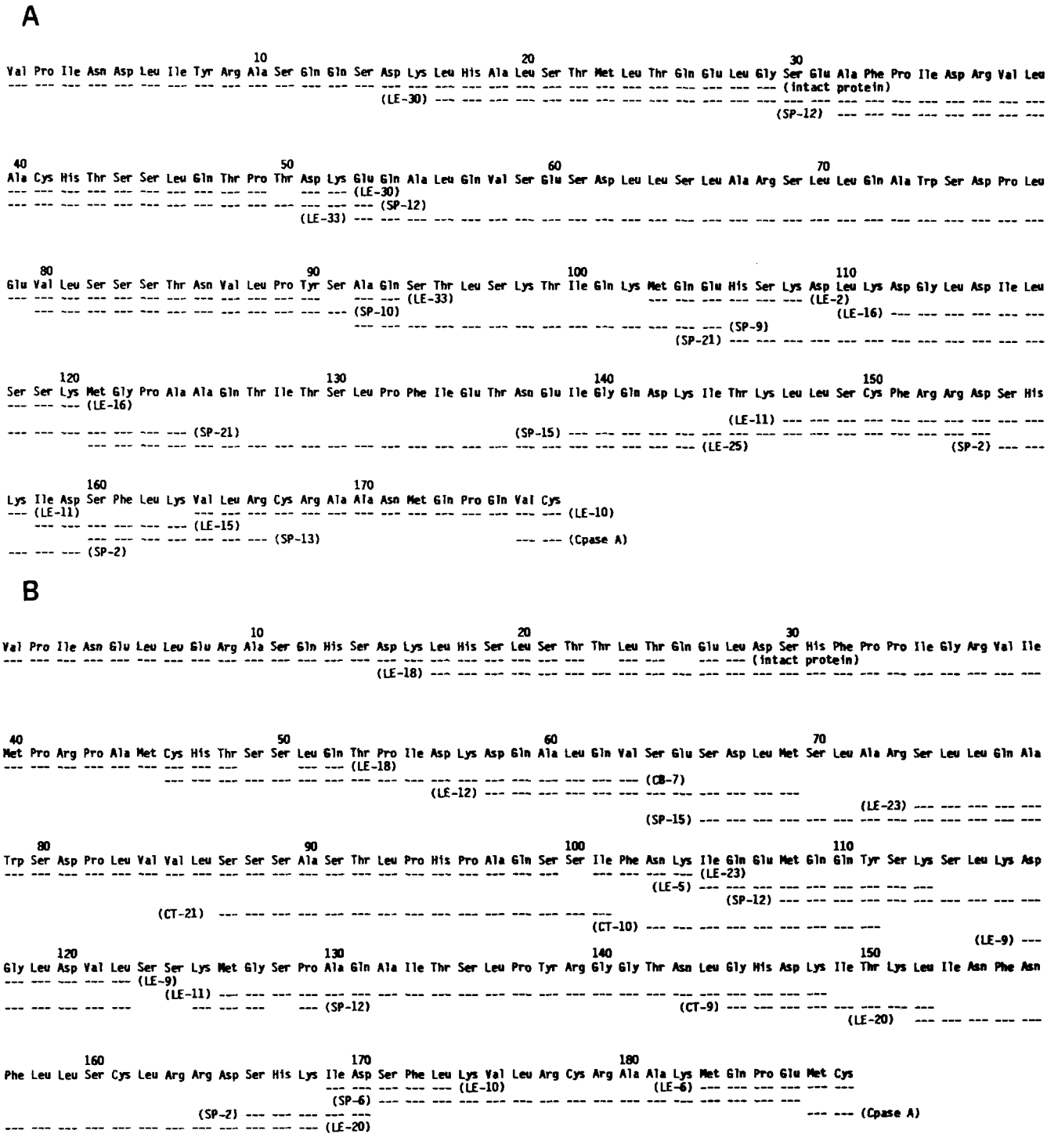

FIG. 1. *A*, complete amino acid sequence of 20-kDa tilapia PRL. *Broken lines* indicate amino acid residues determined by sequence analyses. The designations *LE* and *SP* represent peptides derived by lysyl endopeptidase and *S. aureus* protease, respectively. *Cpase A* represents the sequence derived 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 phenylthiohydantoins were identified by HPLC on a C_8 reverse-phase column (Senshu Kagaku, SEQ-4, C_8, 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

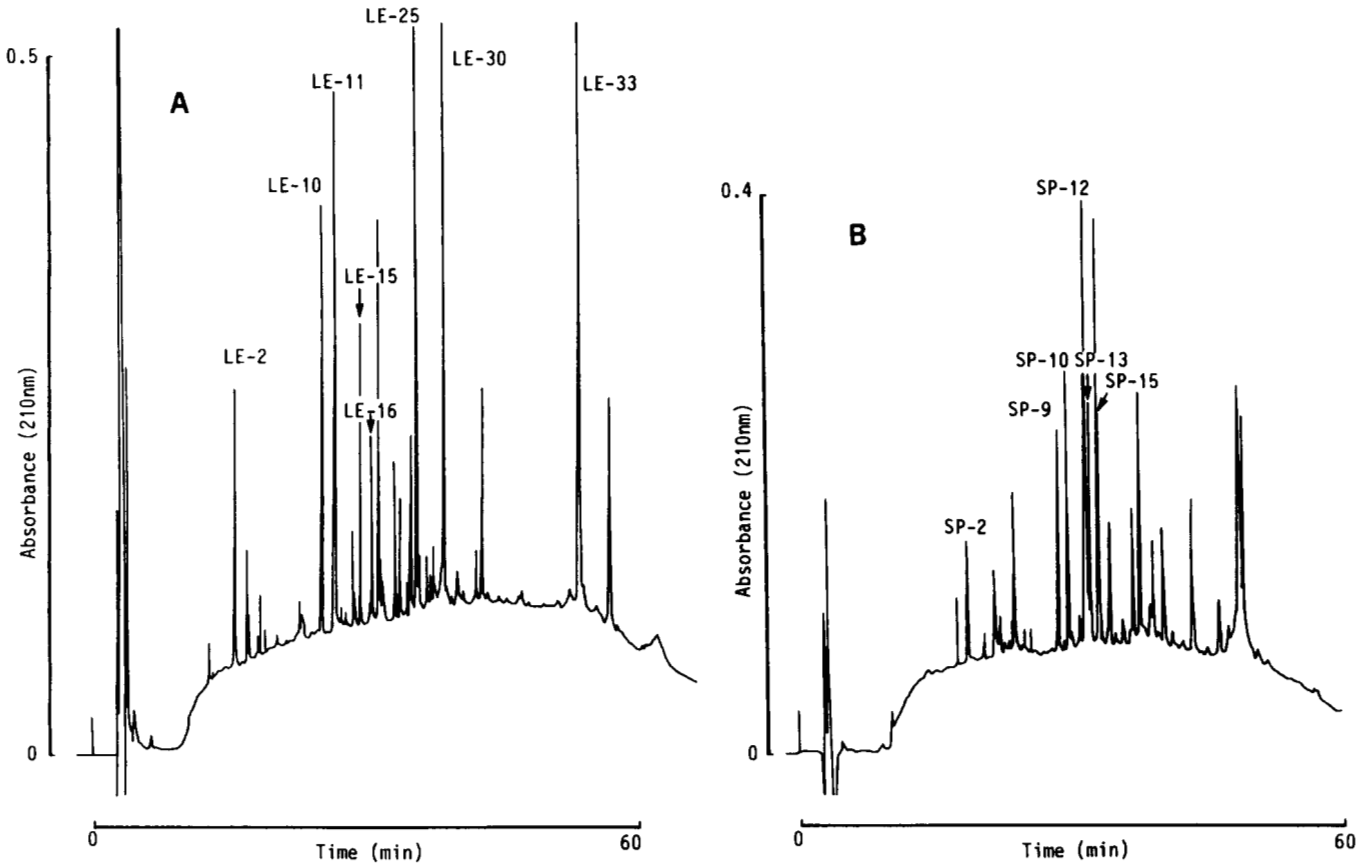

FIG. 2. *A*, separation by HPLC of a lysyl endopeptidase digest of 20-kDa PRL on a YMC AM-312 column (0.6 × 15 cm, particle size 5 μm). Elution was performed with a 60-min linear gradient of 0–70% acetonitrile containing 0.1% trifluoroacetic acid at a flow rate of 1 ml/min. *B*, separation by HPLC of 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. 2*A*) 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. 2*B*). 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 generated 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. 3*A*)) and four *S. aureus* protease peptides (SP-2, SP-6, SP-12, SP-15, Fig. 3*B*) 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.

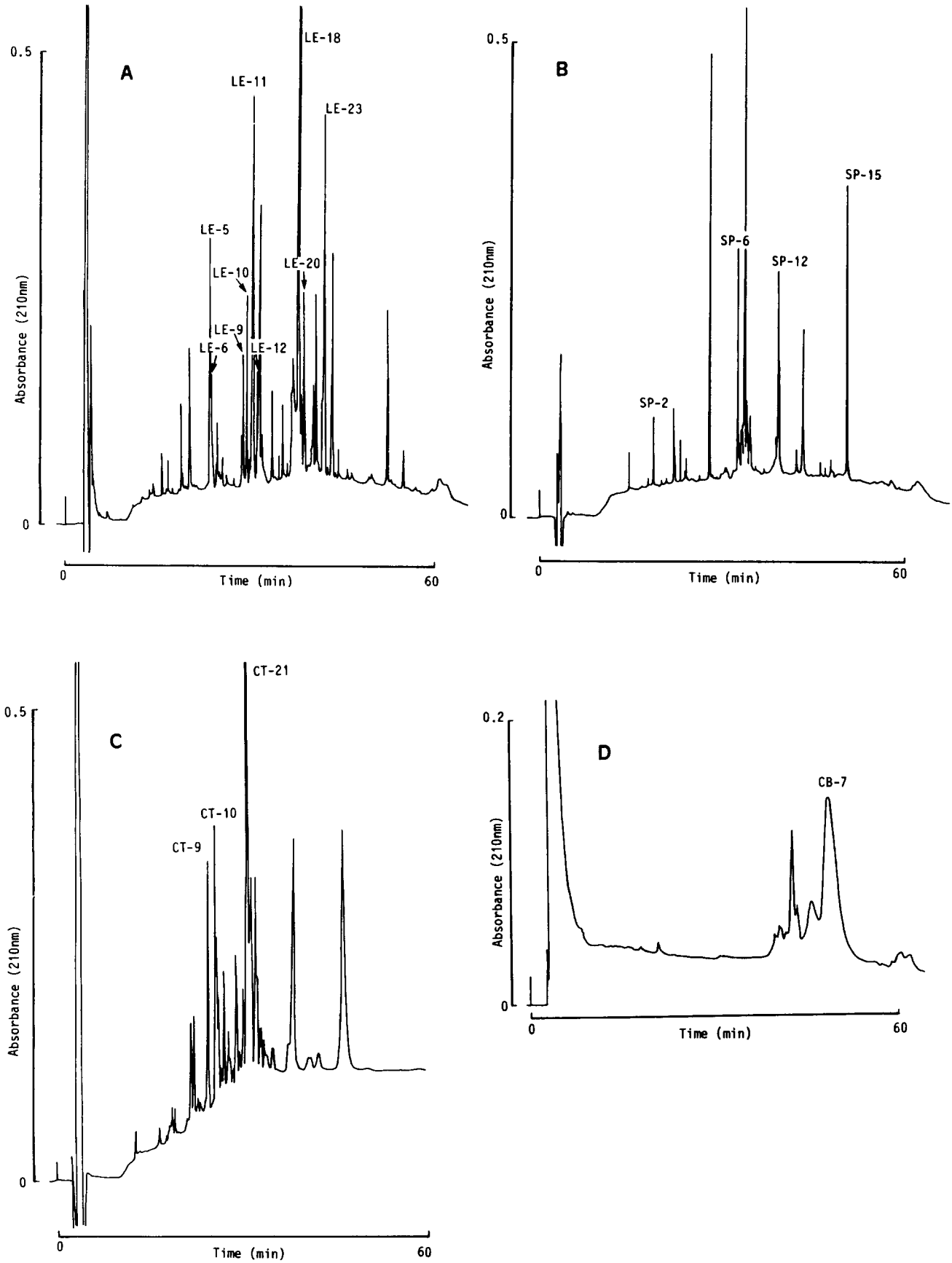

FIG. 3. *A*, separation by HPLC of a lysyl endopeptidase digest of tilapia 24-kDa PRL. Chromatographic conditions are as in Fig. 2*A*. *B*, separation by HPLC of a *S. aureus* protease digest of tilapia 24-kDa PRL. Chromatographic conditions are as in Fig. 2. *C*, separation by HPLC of a chymotrypsin 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. 4*A*). 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.

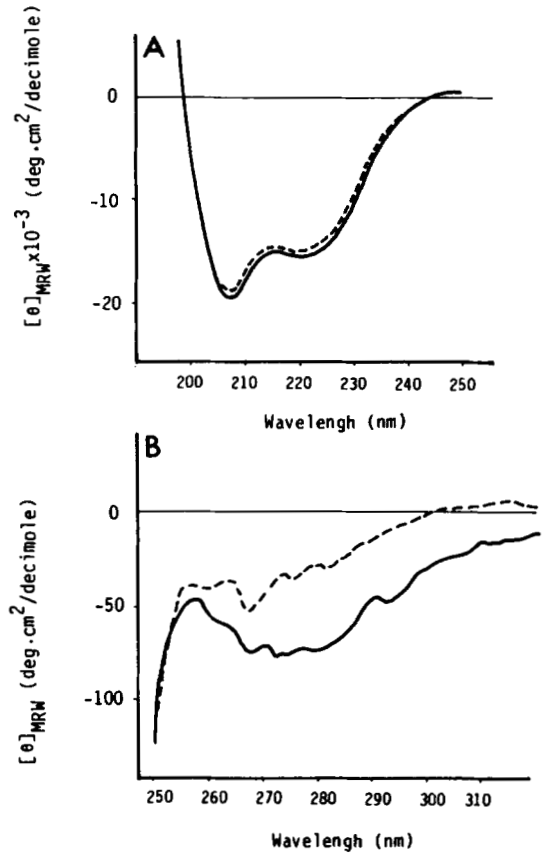

FIG. 4. **Circular dichroism spectra of 20-kDa PRL (——) and the 24-kDa PRL (– – –) in H_2O.** *A*, amide bond circular dichroism spectra. *B*, side-chain circular dichroism spectra.

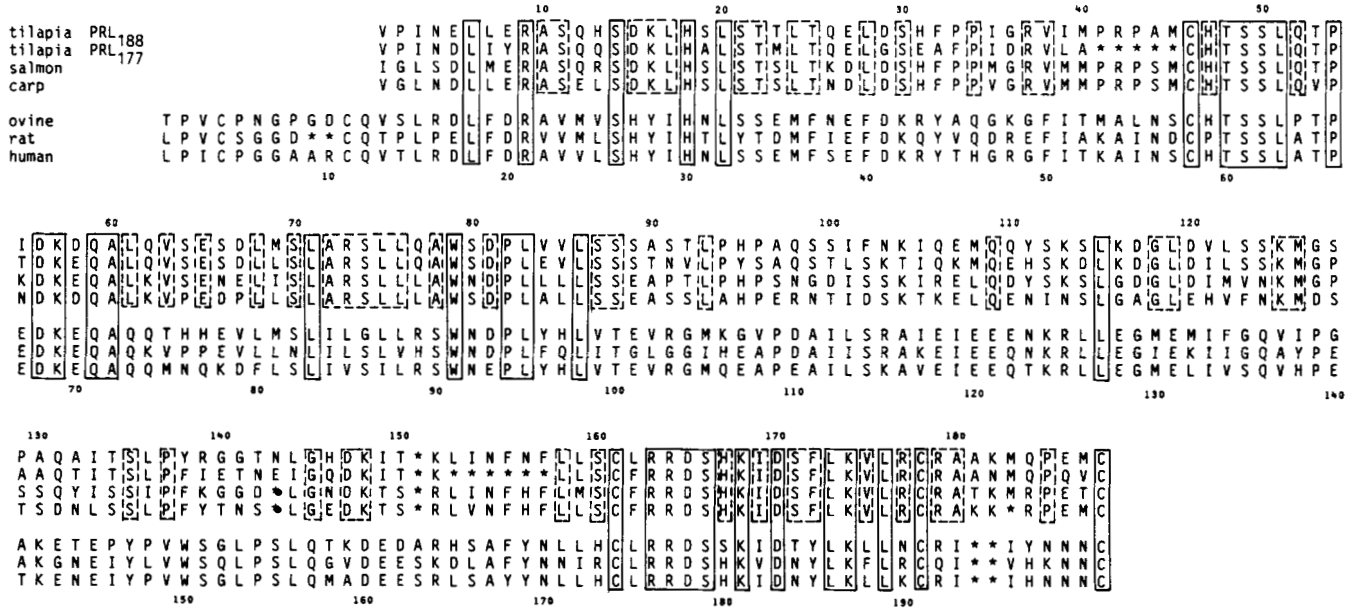

FIG. 5. **Alignment of amino acid sequences (represented by standard single-letter abbreviations) of $tPRL_{177}$ and $tPRL_{188}$ with those of PRLs from salmon (11), carp (12), sheep (20), rat (21), and human (22).** Identical residues among the teleost PRLs are *boxed* with a *broken line*. Identical 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 NH_2-terminal sequence (81%, 21/26) (10). The larger tilapia PRL, previously termed 24-kDa PRL and henceforth termed $tPRL_{188}$, contains 188 residues. The smaller tilapia PRL, previously termed 20-kDa PRL and henceforth called $tPRL_{177}$, contains 177 residues. The more basic isoelectric point of $tPRL_{188}$ (8.7) compared with that of $tPRL_{177}$ (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

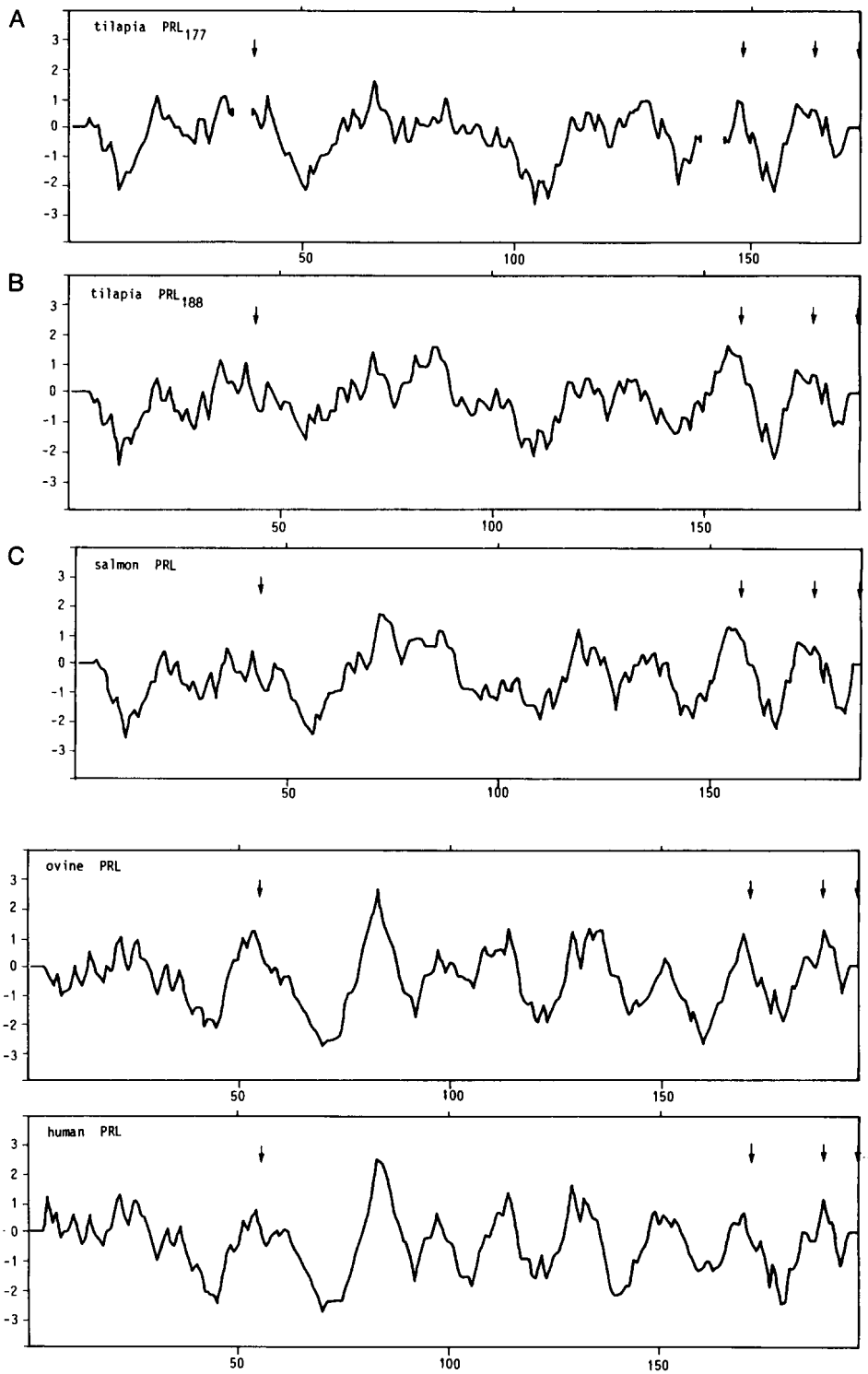

FIG. 6. **Hydropathy profiles of $tPRL_{177}$ (*A*), $tPRL_{188}$ (*B*), salmon PRL (*C*), ovine PRL (*D*), and human PRL (*E*).** A hydropathy score for each residue was obtained by averaging the hydropathy indices (34) of 9 amino acids at a time. *Arrows* indicate the position of cysteine residues.

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. $tPRL_{177}$ is about 56% (105/188) identical to salmon PRL and 51% (96/188) identical to carp PRL. $tPRL_{188}$ 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 $tPRL_{188}$, $tPRL_{177}$ 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 $tPRL_{177}$ 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, $tPRL_{177}$, but not $tPRL_{188}$ 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 $tPRL_{177}$ 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 NH_2-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 hydropathy 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 hydropathy 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 $tPRL_{177}$, both located on the NH_2-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.

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

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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 tPRL177

Position	Residue	Peptide, (cycle), yield, (pmole)									
		Intact Protein									
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	LE-30							
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	SP-12					
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)	6	(17)	15				
49	Pro			(33)	13	(18)	35				
50	Thr			(34)	-	(19)	16				
51	Asp	LE-33		(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)	310								
60	Glu	(8)	165								
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	SP-10							
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			SP-9			
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	LE-2						(10)	178		
102	Lys							(11)	60		
103	Met	(1)	845					(12)	118		
104	Gln	(2)	860	SP-21				(13)	56		
105	Glu	(3)	685					(14)	19		
106	His	(4)	103	(1)	102						
107	Ser	(5)	454	(2)	40						
108	Lys	(6)	295	(3)	65						
109	Asp			(4)	46						
110	Leu	LE-16		(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			LE-25			
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)	116		
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	SP-15						(17)	74		
138	Glu							(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		
142	Lys	(5)	120					(23)	8		
143	Ile	(6)	200								
144	Thr	(7)	54	LE-11							
145	Lys	(8)	106								
146	Leu	(9)	228	(1)	1042						
147	Leu	(10)	222	(2)	912						
148	Ser	(11)	68	(3)	292						
149	Cys	(12)	46	(4)	538						
150	Phe	(13)	132	(5)	656						
151	Arg	(14)	24	(6)	81						
152	Arg	(15)	20	(7)	105			SP-2			
153	Asp	(16)	6	(8)	122						
154	Ser			(9)	168			(1)	132		
156	His	LE-15		(10)	30			(2)	98		
157	Lys			(11)	32			(3)	145		
158	Ile	(1)	1100	SP-13				(4)	210		
159	Asp	(2)	624					(5)	36		
160	Ser	(3)	294	(1)	80						
161	Phe	(4)	745	(2)	370						
162	Leu	(5)	672	(4)	384			LE-10			
163	Lys	(6)	304	(5)	300						
164	Val			(6)	352			(1)	1108		
165	Leu			(7)	365			(2)	1080		
166	Arg			(8)	80			(3)	116		
167	Cys							(4)	442		
168	Arg							(5)	106		
169	Ala							(6)	434		
170	Ala							(7)	392		
171	Asn							(8)	208		
172	Met							(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 tPRL188

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

Table 4. Amino acid sequence data for carboxamidomethylated tPRL188

Position	Residue	Peptide, (cycle), yield, (pmole)							
		Intact Protein							
1	Val	(1)	771						
2	Pro	(2)	383						
3	Ile	(3)	691						
4	Asn	(4)	435						
5	Glu	(5)	323						
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	LE-18					
16	Lys	(16)	197						
17	Leu	(17)	188	(1)	603				
18	His	(18)	20	(2)	246				
19	Ser	(19)	42	(3)	262				
20	Leu	(20)	166	(4)	338				
21	Ser	(21)	33	(5)	170				
22	Thr	(22)	23	(6)	35				
23	Thr	(23)	-	(7)	44				
24	Leu	(24)	115	(8)	275				
25	Thr	(25)	10	(9)	31				
26	Gln	(26)	-	(10)	240				
27	Glu	(27)	60	(11)	193				
28	Leu	(28)	110	(12)	195				
29	Asp			(13)	143				
30	Ser			(14)	58				
31	His			(15)	40				
32	Phe			(16)	107				
33	Pro			(17)	110				
34	Pro			(18)	170				
35	Ile			(19)	113				
36	Gly			(20)	77				
37	Arg			(21)	41				
38	Val			(22)	87				
39	Ile			(23)	112				
40	Met			(24)	60				
41	Pro			(25)	28				
42	Arg			(26)	28				
43	Pro			(27)	90				
44	Ala			(28)	30	CB-7			
45	Met			(29)	25				
46	Cys			(30)	17	(1)	70		
47	His			(31)	9	(2)	43		
48	Thr			(32)	7	(3)	12		
49	Ser			(33)	-	(4)	49		
50	Ser			(34)	-	(5)	58		
51	Leu			(35)	14	(6)	113		
52	Gln			(36)	11	(7)	67		
53	Thr					(8)	16		
54	Pro					(9)	76		
55	Ile					(10)	71		
56	Asp	LE-12				(11)	80		
57	Lys					(12)	59		
58	Asp	(1)	36			(13)	85		
59	Gln	(2)	56			(14)	72		
60	Ala	(3)	45			(15)	56		
61	Leu	(4)	66			(16)	105		
62	Gln	(5)	33			(17)	75		
63	Val	(6)	44			(18)	59		
64	Ser	(7)	15	SP-15					
65	Glu	(8)	23						
66	Ser	(9)	16	(1)	62				
67	Asp	(10)	18	(2)	67				
68	Leu	(11)	24	(3)	110				
69	Met	(12)	6	(4)	82				
70	Ser			(5)	35				
71	Leu			(6)	79				
72	Ala	LE-23		(7)	86				
73	Arg			(8)	22				
74	Ser	(1)	186	(9)	22				
75	Leu	(2)	431	(10)	46				
76	Leu	(3)	430	(11)	58				
77	Gln	(4)	365	(12)	25				
78	Ala	(5)	258	(13)	42				
79	T p	(6)	58						
80	Ser	(7)	63						
81	Asp	(8)	202						
82	Pro	(9)	175						
83	Leu	(10)	160						
84	Val	(11)	128						
85	Val	(12)	160	CT-21					
86	Leu	(13)	105						
87	Ser	(14)	56	(1)	378				
88	Ser	(15)	58	(2)	283				
89	Ser	(16)	47	(3)	317				
90	Ala	(17)	51	(4)	398				
91	Ser	(18)	25	(5)	117				
92	Thr	(19)	10	(6)	38				
93	Leu	(20)	26	(7)	218				
94	Pro	(21)	35	(8)	216				
95	His	(22)	9	(9)	114				
96	Pro	(23)	24	(10)	178				
97	Ala	(24)	11	(11)	145				
98	Gln	(25)	16	(12)	198				
99	Ser	(26)	13	(13)	70				
100	Ser	(27)	-	(14)	41				
101	Ile	(28)	11	(15)	10	CT-10			
102	Phe	(29)	11						
103	Asn	(30)	6	LE-5		(1)	762		
104	Lys	(31)	6			(2)	556		
105	Ile			(1)	410	(3)	671		
106	Gln	SP-12		(2)	420	(4)	578		
107	Glu			(3)	520	(5)	560		
108	Met	(1)	60	(4)	388	(6)	630		
109	Gln	(2)	62	(5)	385	(7)	381		
110	Gln	(3)	54	(6)	447	(8)	380		
111	Tyr	(4)	34	(7)	484	(9)	179		
112	Ser	(5)	20	(8)	91				
113	Lys	(6)	47	(9)	58				
114	Ser	(7)	19						
115	Leu	(8)	45	LE-9					
116	Lys	(9)	52						
117	Asp	(10)	24	(1)	306				
118	Gly	(11)	18	(2)	193				
119	Leu	(12)	40	(3)	170				
120	Asp	(13)	41	(4)	211				
121	Val	(14)	33	(5)	177				
122	Leu	(15)	28	(6)	186				
123	Ser	(16)	-	(7)	40				
124	Ser	(17)	-	(8)	45	LE-17			
125	Lys	(18)	16	(9)	60				
126	Met	(19)	18			(1)	521		
127	Gly	(20)	19			(2)	573		
128	Ser	(21)	-			(3)	102		
129	Pro	(22)	12			(4)	467		
130	Ala					(5)	504		
131	Gln					(6)	404		
132	Ala					(7)	401		
133	Ile					(8)	266		
134	Thr					(9)	125		
135	Ser					(10)	28		
136	Leu					(11)	168		
137	Pro					(12)	198		
138	Tyr					(13)	215		
139	Arg					(14)	71		
140	Gly					(15)	189		
141	Gly					(16)	198		
142	Thr					(17)	47		
143	Asn	CT-9				(18)	114		
144	Leu					(19)	136		
145	Gly	(1)	580			(20)	102		
146	His	(2)	298			(21)	26		
147	Asp	(3)	559			(22)	98		
148	Lys	(4)	677			(23)	16		
149	Ile	(5)	501						
150	Thr	(6)	26	LE-20					
151	Lys	(7)	474						
152	Leu	(8)	162	(1)	122				
153	Ile			(2)	97				
154	Asn			(3)	156				
155	Phe			(4)	119				
156	Asn			(5)	110				
157	Phe			(6)	90				
158	Leu			(7)	108				
159	Leu			(8)	135				
160	Ser			(9)	40				
161	Cys			(10)	58				
162	Leu			(11)	51				
163	Arg			(12)	44				
164	Arg			(13)	52	SP-2			
165	Asp			(14)	105				
166	Ser			(15)	22	(1)	120		
167	His	LE-10		(16)	30	(2)	103		
168	Lys			(17)	11	(3)	160		
169	Ile	(1)	263	SP-6		(4)	241		
170	Asp	(2)	275			(5)	60		
171	Ser	(3)	45	(1)	58				
172	Phe	(4)	128	(2)	355				
173	Leu	(5)	100	(3)	366				
174	Lys	(6)	37	(4)	314				
175	Val			(5)	332				
176	Leu			(6)	215				
177	Arg			(7)	96				
178	Cys			(8)	233				
179	Arg			(9)	95				
180	Ala			(10)	210				
181	Ala	LE-6		(11)	169				
182	Lys			(12)	110				
183	Met	(1)	1002	(13)	232				
184	Gln	(2)	843	(14)	171				
185	Pro	(3)	451	(15)	138				
186	Glu	(4)	625	(16)	21				
187	Met	(5)	319	(17)	8				
188	Cys	(6)	166						

Table 5. Carboxypeptidase A digestion of carboxamidomethylated tPRL188

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