Proc. Natl. Acad. Sci. USA
Vol. 82, pp. 1050–1053, February 1985
Biochemistry

Parathymosin α: A peptide from rat tissues with structural homology to prothymosin α

(thymic peptide/tissue immunomodulator/immunomodulating peptide/protection against *Candida albicans*)

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ABSTRACT A peptide, parathymosin α, containing ≈105 amino acid residues, has been isolated from rat thymus, and the sequence of the first 30 residues at the NH_2 terminus has been determined. In this region, it shows 43% structural identity with thymosin α_1 and prothymosin α. The common sequences do not include residues 2–9, which accounts for the poor reactivity of parathymosin α with an antibody directed against this epitope in thymosin α_1. Parathymosin α appears to modulate the action of prothymosin α in protecting sensitive strains of mice against opportunistic infection with *Candida albicans*.

We have recently reported (1) a procedure for the isolation from rat thymus of three previously identified peptides, thymosin β_4 (2), thymosin β_{10} (3), and prothymosin α (4, 5). Prothymosin α, identified as a larger polypeptide containing the thymosin α_1 sequence (6) at its NH_2 terminus, was shown to account for most, if not all, of the immunoreactivity detected with an antibody directed against the NH_2-terminal sequence in thymosin α_1 (4).

In this paper, we report that the isolation procedure also yields a fourth peptide, named parathymosin α because it shows some structural homology to prothymosin α and because it has a similar size and amino acid composition. Parathymosin α can also be isolated from other rat tissues, and in some tissues, such as liver, kidney, and brain, the concentrations of parathymosin α are much higher than the concentrations of prothymosin α. Preliminary results suggest that parathymosin α may act to modulate the immunoenhancing activity exhibited by prothymosin α.

EXPERIMENTAL PROCEDURES

Materials. Unless otherwise indicated, materials used were as described (1, 4, 5).

Methods. The procedures for processing and extracting tissues and separating peptides from the extracts were as described (4, 5). Briefly, fresh rat thymuses were frozen in liquid N_2 and pulverized in the frozen state. The powdered frozen tissue was quickly brought to 95°C–100°C in a relatively large volume of boiling 0.1 M sodium phosphate buffer (pH 7.0) and, after cooling, the suspension was homogenized with a Polytron homogenizer (Brinkmann) and the soluble fraction was collected. The clear extracts were desalted on Sep-Pak C-18 cartridges (Waters Associates), and the peptides were eluted with 1 M HCOOH/0.2 M pyridine, separated by chromatography on a column of Sephacryl S-200, and purified by reversed-phase HPLC (1, 4).

Amino acid analyses, automated sequencing of peptides, and isoelectric focusing were carried out as described (4).

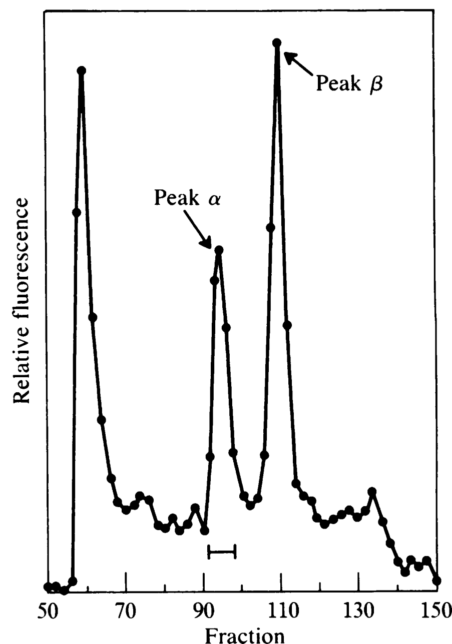

FIG. 1. Sephacryl S-200 gel filtration of peptides extracted from rat thymus. Aliquots (0.8 ml) of the lyophilized eluates from the Sep-Pak C-18 cartridges, dissolved in 1 M HCOOH/0.2 M pyridine, pH 2.8 (buffer A), were applied to a Sephacryl S-200 (1.5 × 89 cm) column, previously equilibrated with buffer A. The column was developed with the same buffer at a flow rate of 8.5 ml/hr, and 0.85-ml fractions were collected. To locate peptide peaks, aliquots (10 μl) were dried, hydrolyzed with alkali, and analyzed with fluorescamine (4). For subsequent purification by HPLC, the fractions corresponding to peak α, as indicated by the bar, were pooled and combined with similar fractions from three other gel filtration separations.

The mouse protection assay was carried out with susceptible strains of mice challenged with *Candida albicans*, as described by Salvin and Neta (7, 8).

RESULTS

Purification of Parathymosin α from Extracts of Rat Thymus. Chromatography of the desalted extracts on Sephacryl S-200 yielded three peaks (Fig. 1). Further separation of the peptides in peak β by HPLC showed that this fraction contained thymosins β_4 and β_{10} as the major components (1). Peak α was found to contain prothymosin α (peak 2 in Fig. 2*A*; see also ref. 4) and a second peptide (peak 3 in Fig. 2*A*),

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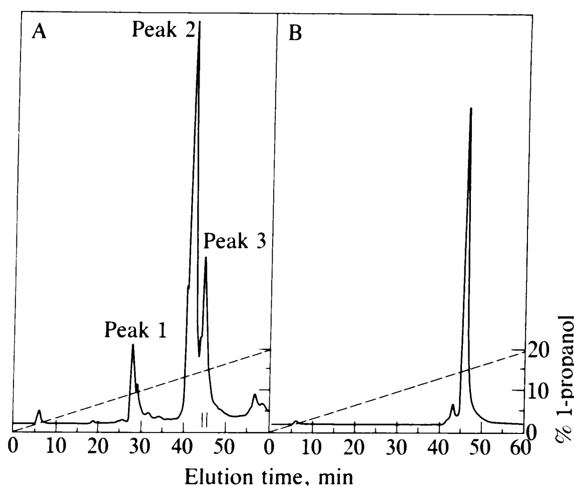

FIG. 2. Isolation of parathymosin α by separation of peptides in peak α by reversed-phase HPLC. (*A*) Fractions comprising peak α (pooled as described in the legend to Fig. 1) were lyophilized and the residue was dissolved in 900 μl of buffer A. HPLC experiments were carried out with 150-μl aliquots of this solution (4). Elution was with a gradient of 0–20% 1-propanol in buffer A as shown (- - -). Fractions (0.6 ml) were collected. For analysis with fluorescamine, 5-μl aliquots were diverted to the detector every 6 sec. In the experiment shown, fractions 45 and 46 (peak 3) were pooled, combined with similar fractions from five other HPLC separations, and lyophilized. (*B*) Lyophilized peptides from peak 3 (57 μg) (see *A*) were chromatographed on reversed-phase HPLC under the conditions described above. Major protein peak represents parathymosin α.

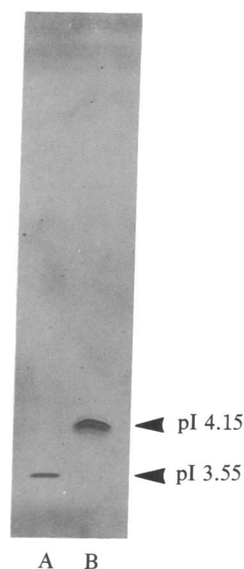

FIG. 3. Analytical isoelectric focusing of prothymosin α (lane A) and parathymosin α (lane B). Aliquots of each (25 and 30 μg, respectively) were applied to an LKB Ampholine polyacrylamide gel plate and developed as described (4).

which was purified by HPLC to yield pure parathymosin α (Fig. 2*B*).

Characterization of Parathymosin α. The amino acid composition of parathymosin α was similar to that previously reported for prothymosin α (Table 1), the major differences being (*i*) a smaller number of aspartyl (or asparaginyl) residues, (*ii*) the higher content of basic amino acids, and (*iii*) the absence of isoleucine. The differences in amino acid composition account for the higher isoelectric point of parathymosin α (pI, 4.15), as compared with prothymosin α (pI, 3.55; Fig. 3).

Distribution and Tissue Content of Parathymosin α. When the isolation procedure was applied to extracts from other rat tissues, such as lung, kidney, liver, and brain, the HPLC peak corresponding to parathymosin α was found to be much more prominent than the peak containing prothymosin α (see figure 2 in ref. 5). Amino acid analysis of the peptides in this peak confirmed their identity with parathymosin α purified from rat thymus (Table 2).

The concentrations of parathymosin α in rat tissues were estimated from the relative peak heights of peaks 2 and 3 (figure 2 in ref. 5) after separation by HPLC and the known quantities of prothymosin α in the crude tissue extracts, which were determined by a radioimmunoassay (5) using purified prothymosin α as the reference standard (Table 3). In contrast to thymus and spleen, in which prothymosin α was the major peptide, the concentration of parathymosin α was found to be highest in rat liver, followed by kidney, lung, and brain. The reciprocal relationship between the concentrations of prothymosin α and parathymosin α resulted in relatively constant values for the sum of the concentrations of the two peptides. The low value for the total estimated for

Table 1. Amino acid composition of parathymosin α

Residue	Parathymosin α	Prothymosin α
Asx	13.0 ± 0.8 (13)	24
Thr	2.8 ± 0.1 (3)	6
Ser	3.7 ± 0.2 (4)	3
Glx	39.5 ± 1.6 (40)	39
Gly	6.5 ± 0.2 (7)	10
Ala	10.8 ± 0.5 (11)	10
Val	4.5 ± 0.1 (5)	6
Ile	0.05 ± 0.01 (0)	1
Leu	2.0 ± 0.0 (2)	1
Lys	12.5 ± 0.3 (13)	9
Arg	4.7 ± 0.1 (5)	2
Pro	1.8 ± 0.0 (2)	2
Total	(105)	113

Parathymosin α values shown are mean ± SD from analyses of six samples of parathymosin α hydrolyzed with redistilled 5.7 M HCl at 150°C for 1 hr. Values were calculated assuming two residues of leucine in parathymosin α and one residue of leucine in prothymosin α. The nearest integral numbers are shown in parentheses. Tryptophan, cysteine, phenylalanine, tyrosine, histidine, and methionine were not detected. Prothymosin α values are based on results of sequence determination (9).

Table 2. Amino acid compositions of parathymosin α from various rat tissues

Residue	Thymus	Spleen	Lung	Kidney	Liver	Brain
Asx	13.0	13.8	13.3	14.3	13.1	13.4
Thr	2.8	3.2	3.2	3.1	2.7	2.7
Ser	3.7	3.8	3.9	3.8	3.7	3.7
Glx	39.5	38.2	42.3	41.0	38.2	37.6
Gly	6.5	7.2	6.6	6.9	6.6	7.0
Ala	10.8	9.5	8.7	10.9	10.1	10.9
Val	4.5	4.4	4.5	4.9	4.6	4.7
Leu	2.0	2.0	2.0	2.0	2.0	2.0
Lys	12.5	12.3	12.5	13.7	12.4	13.1
Arg	4.7	4.5	5.0	5.5	4.9	5.0
Pro	1.8	2*	2*	2*	2*	2*

Fractions corresponding to peak 3 from rat thymus (Fig. 2; see also ref. 5) were pooled and aliquots were hydrolyzed with redistilled 5.7 M HCl at 150°C for 1 hr. Amino acid analyses were carried out as described (4). Values were calculated assuming two residues of leucine in parathymosine α.
*Not determined. Content of proline in parathymosin α isolated from these tissues was assumed to be 2.

Table 3. Content of parathymosin α and prothymosin α in rat tissues

Tissue	Peak height ratio*	Prothymosin α,† μg per g of tissue	Parathymosin α,‡ μg per g of tissue	Parathymosin α and prothymosin α, μg per g of tissue
Thymus	0.38	414	157	571
Spleen	0.44	270	119	389
Lung	1.72	154	265	419
Kidney	2.50	126	315	441
Liver	4.80	68	326	394
Brain	3.57	58	207	265

*Estimated from peak heights for prothymosin α and parathymosin α in the HPLC separations, as reported in ref. 5 and from unpublished results for rat liver.
†Based on radioimmunoassay of extracts of boiled tissue (5).
‡Content of parathymosin α is calculated from ratio of peak heights and RIA value for prothymosin α.

brain may be due to uncertainty in the calculation based on the low recovery of prothymosin α in the HPLC separations (5).

NH_2-Terminal Sequence of Parathymosin α. The NH_2 terminus of parathymosin α was found to be blocked, based on the results of automated sequence analysis. The sequence of the first 30 amino acid residues was deduced from the amino acid composition, and sequence analysis of peptides was obtained by digestion with thermolysin (Fig. 4). Identity with prothymosin α was found at positions 1, 5, 10, 14–20, and 23–25, accounting for 13 of the first 30 residues from the NH_2 terminus.

Absence of Cross-Reactivity with Thymosin α_1 Antiserum. The fractions containing parathymosin α failed to show significant activity in the radioimmunoassay for thymosin α_1, (figure 2 in ref. 4). This can now be attributed to the differences in amino acid sequence at the NH_2 terminus, because the major epitope for the antiserum used was derived from the NH_2-terminal sequence (unpublished results; see also ref. 4).

Biological Activity of Parathymosin α. The cellular functions of prothymosin α and parathymosin α remain unknown. In a mouse protection test (7) previously used to evaluate the immunoenhancing properties of thymosin α_1 and thymosin α_{11} (8), prothymosin α was found to be active (Table 4) at doses significantly lower than those required for the smaller peptide fragments (8), suggesting that its activity was not due to the formation of these fragments. In this mouse protection test, parathymosin α exhibited much weaker activity, but when it was administered together with prothymosin α, it appeared to block the immunoenhancing effects of the latter.

Table 4. Effects of prothymosin α and parathymosin α on the growth of *C. albicans* in C_3H/HeJ mice

Peptide		*C. albicans* cell count	
Prothymosin α, ng	Parathymosin α, ng	Day 12	Day 14
0	0	3740	6670
80	0	0	160
160	0	1	16
320	0	1	1
0	80	1570	2570
0	160	5040	2990
0	320	4270	3510
160	160	820	3860

Mice were treated daily with the indicated doses of prothymosin α and/or parathymosin α. Two days after the start of treatment the mice were challenged with 4×10^4 cells of *C. albicans* (7, 8). Three mice from each set were sacrificed on days 12 and 14 after infection. Cell count values represent the average number of organisms in the left kidneys of the mice in each set.

DISCUSSION

The presence in a variety of rat tissues of a peptide showing significant structural homology with prothymosin α, the major immunoreactive form of thymosin α_1 in rat tissues (4, 5), raises important questions regarding the biological function(s) and interaction of these peptides. Their ubiquitous distribution and relatively high tissue content (ref. 5 and this work) make it unlikely that they function as thymic hormones or lymphokines, as has been proposed for thymosin α_1 (10). The larger peptides may function as precursors of the smaller fragments, such as thymosin α_1 and thymosin α_{11} (8). However, these fragments have not been detected in extracts of tissues prepared as described here (5), but they may account for the immunoreactive substances assayed in circulating blood (11). On the other hand, the fact that prothymosin α is active in the mouse protection test at lower concentrations than thymosin α_1 and thymosin α_{11} suggests that the biologically active forms are the larger peptides, possibly serving as cytokines that are released following tissue or cellular injury. In this connection, the presence in nonlymphoid tissues of higher concentrations of parathymosin α and the fact that it suppresses the immunoenhancing effect of prothymosin α are of particular interest.

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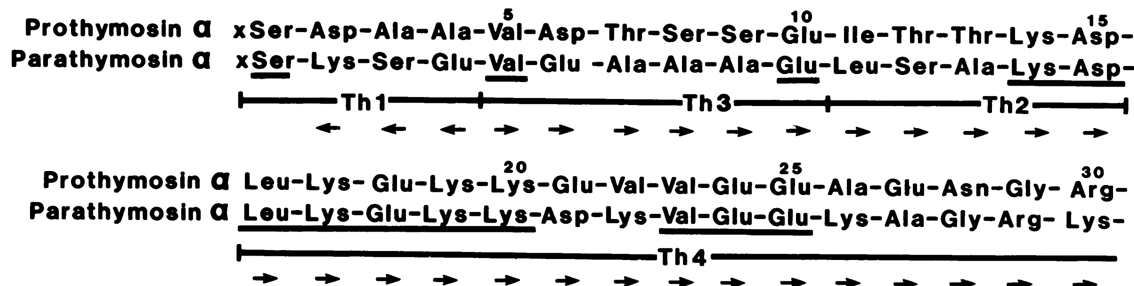

FIG. 4. Amino acid sequence of NH_2-terminal regions of prothymosin α and parathymosin α. Peptides obtained by digestion of parathymosin α with thermolysin were separated by HPLC (see text) and sequences were deduced from results of automated sequence analysis (→) or from digestion with carboxypeptidase A (←). The sequence of the COOH-terminal segment of prothymosin α (4) is shown on the top line, and the identical residues in parathymosin α are underlined.

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