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Thymic Humoral Factor γ2: Purification and Amino Acid Sequence of an Immunoregulatory Peptide from Calf Thymus

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ABSTRACT: Thymic humoral factor γ2 (THF-γ2), an octapeptide essential for immune regulation, was purified from calf thymus. The purification of THF-γ2, monitored in vitro and in vivo in mouse splenocyte proliferation assays, was achieved by gel filtration of low molecular weight thymus extracts followed by ion-exchange chromatography and sequential reversed-phase high-performance liquid chromatography. The process yielded 5 μg of THF-γ2/1000 kg of thymus tissue. The concentration of THF-γ2 required for augmentation of lymphocyte proliferation and interleukin 2 production was 5 ng/mL in vitro and 10 ng/kg per mouse in vivo. THF-γ2 has the amino acid sequence Leu-Glu-Asp-Gly-Pro-Lys-Phe-Leu. The proposed structure has been confirmed because a peptide was synthesized on the basis of this sequence that showed activity identical with that of the biological molecule. It shows no homology to the amino acid sequence of other thymic hormones nor is it part of any peptide or protein of known sequence. THF-γ2 retains essentially all of the biological activity of the thymus extract from which it is derived.

The immunological importance of the endocrine thymus is well established (Bach, 1976). The view that thymic hormones play a critical role in the regulation of immunocompetence developed from observations by us and others that implantation of thymus grafts in cell-impermeable diffusion chambers (Levey et al., 1963a,b; Osoba & Miller, 1963) or injection of thymus extracts (Small & Trainin, 1967; Hand et al., 1970; Trainin & Linker-Israeli, 1967) into neonatally thymectomized (NTx)[1] mice could reconstitute the resultant profound deficit in humoral and cell-mediated immunity. Previous research in our laboratories has demonstrated that in animal models, a crude extract of calf thymus denoted thymic humoral factor (THF) is essential for induction of clonal expansion, differentiation, and maturation of T-cell subsets (Kook & Trainin, 1974; Trainin et al., 1975, 1985). THF augmented most T-cell functions, such as the response to T-cell lectins, mixed lym-

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[1] Abbreviations: THF, thymic humoral factor; T-cell, thymus-derived lymphocytes; TFA, trifluoroacetic acid; IL-2, interleukin 2; Con A, concanavalin A; PHA, phytohemagglutinin; MLR, mixed lymphocyte reaction; NTx, neonatally thymectomized; HPLC, high-performance liquid chromatography; PTH, phenylthiohydantoin; Hepes, *N*-(2-hydroxyethyl)piperazine-*N*′-2-ethanesulfonic acid; PBS, phosphate-buffered saline; FCS, fetal calf serum; Da, dalton(s); FTS, serum thymic factor; Tris-HCl, tris(hydroxymethyl)aminomethane hydrochloride.

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phocyte reactions, graft versus host reactivity, and cytotoxic responses both in thymus-deprived and in intact mice. Clinical data have supported the notion that in humans, THF regulates differentiation of T-cell precursors, leading to normalization of impaired ratios between helper (CD4+) and suppressor/cytotoxic (CD8+) subsets (Trainin et al., 1985, 1986; Handzel et al., 1985, 1987). Administration of THF was followed by reconstitution of defective cell-mediated immunity in patients affected by various types of neoplasms and suffering from secondary immune deficiency as a result of chemo- and/or radiotherapy, many in association with severe viral infections including measles, adenovirus, herpes, varicella, and cytomegalovirus (Trainin et al., 1985, 1986; Handzel et al., 1985, 1987; unpublished observations). Although these studies were uncontrolled in the majority of the patients, a repeatable pattern, suggestive of cause and effect, emerged in most of the cases, namely, rapid regression of the viral infection and reconstitution of cell-mediated immune parameters after a few days of THF treatment.

Isolation of the active principal of THF has been arduous because of its high specific activity and the difficulties inherent in bioassays. Since even a minimal contaminant could account for all the bioactivity of an apparently chemically pure material, confirmation in vivo with a chemically synthesized molecule is the only final proof of relevance of a determined structure to its claimed activity. We now report the purification to homogeneity and the amino acid sequence of an octapeptide, designated THF-γ2, which retains essentially all the biological properties of the thymus extract from which it derived. The chemical synthesis of THF-γ2, using a solid-phase procedure, has been accomplished (unpublished data). The synthetic material is identical with biological THF-γ2 in physicochemical properties and contains all its biological activities, thus confirming the proposed primary structure.

Experimental Procedures

Source of THF-γ2. THF-γ2 was purified from crude THF, which was isolated from calf thymus by a modification of the procedure of Kook et al. (1975). DEAE-Sephadex A-25 and Sephadex G-10 and G-25 were from Pharmacia (Uppsala, Sweden). Bio-Gel P-2 was from Bio-Rad (Richmond, CA). Lichrosorb RP-18 (10 μm) powder was from Merck (Darmstadt, West Germany). Prepacked 200 × 4 mm HPLC columns containing Nucleosil (5 μm) were from Macherey–Nagel (Düren, West Germany). All reagents were of analytical grade; HPLC solvents were HPLC grade with the exception of 1-propanol, which was freshly redistilled.

Reversed-Phase High-Performance Liquid Chromatography. The final purification of THF-γ2 was achieved by reversed-phase HPLC. All peptide separations were performed with Spectra-Physics SP8100 and SP8750 liquid chromatography systems equipped with SP8440 and SP8733 variable-wavelength detectors. The column effluents were monitored by UV absorbance at 280, 220, and 210 nm, and fractions were assayed for biological activity. At each purification step, fractions showing activity in the PHA, Con A, and MLR bioassays were submitted to confirmation of clinical efficacy (Trainin et al., 1985, 1986; Handzel et al., 1985, 1987).

Molecular Weight Determination. The apparent molecular weight of THF-γ2 was determined by high-performance gel permeation chromatography, using a column of TSK-2000SW (Varian, Walnut Creek, CA). Molecular weight calibration standards included cytochrome *c* (12 500), insulin (5700), and bacitracin (1450).

Amino Acid Composition Analysis and Sequence Determination. For amino acid composition analysis, samples were hydrolyzed in 6 N HCl at 110 °C for 22 h under vacuum and analyzed with a Biotronik Model LC6001 automatic amino acid analyzer. Amino acid sequence analysis of THF-γ2 was performed by using a Model 470A gas-phase microsequencer equipped with an on-line phenylthiohydantoin analyzer, Model 120A (Applied Biosystems, Foster City, CA). A total of 3.0 mg of BioBrene Plus (Applied Biosystems) was applied and subjected to three precycles of Edman degradation prior to sample application. Conversion of the thiazolinone derivatives to phenylthiohydantoin-amino acids was carried out with 25% TFA. Phenylthiohydantoin-amino acid derivatives were separated by reversed-phase HPLC on a PTH C_{18} column (2.1 × 220 mm; Applied Biosystems) with a sodium acetate buffer/tetrahydrofuran/acetonitrile gradient (Hunkapiller & Hood, 1983), on-line, on a Model 120A analyzer (Applied Biosystems).

Bioassays. Bioassays of THF activity have been described previously. The proliferative response of T-lymphocytes in vitro to stimulation by the lectins PHA (phytohemagglutinin) and Con A was performed according to Rotter and Trainin (1975) to pokeweed mitogen according to Handzel et al. (1987) and to allogeneic cells in a one-way mixed lymphocyte reaction (MLR) as described by Umiel and Trainin (1975). Injection of THF into NTx mice reconstitutes the ability of spleen cells to react in the above assays. Since augmentation of mitogen proliferation alone was considered to be nonspecific, all three parameters, PHA, Con A, and MLR, both in vitro and in vivo, were used to monitor the purification process and denoted the "requisite" bioassays. Cyclic AMP activity was performed as described by Kook and Trainin (1974). This activity, which was present only in crude THF preparations, was included in the battery of assays during initial purification steps but was not used as a criterion for final purification of THF-γ2.

Confirmation of clinical efficacy was performed in several hospitals in Israel, under the supervision of Drs. R. Zaizov and Z. T. Handzel. Different THF preparations were administered to patients suffering from secondary immune deficiency as a result of anticancer chemoradiotherapy, as described elsewhere (Handzel et al., 1985, 1987; Trainin et al., 1986).

Production of Interleukin 2 (IL-2). Spleens of mice to be assayed were aseptically removed, and single-cell suspensions were prepared by standard procedures. For culture, 10^7 cells/mL were incubated at 37 °C in 5% CO_2/95% air, in 24-well Costar plates containing RPMI-1640 medium supplemented with 20 mM Hepes buffer and 50 μM 2-mercaptoethanol. Con A (Bio-Makor, Rehovot, Israel) was added at 2.5 μg/mL to a final volume of 1 mL, and after 3 h, the culture medium was replaced by 1 mL of fresh medium. After an additional 21 h, cell-free supernatants (containing IL-2) were harvested by centrifugation and stored at −20 °C.

For assessment of in vitro THF-γ2 activity, spleen cells (10^7 cells/mL, as above) from (C3HeB/Fe × C57Bl/6J)F_1 mice were pretreated with various concentrations (75–600 ng/mL) of THF-γ2 overnight before exposure to Con A, and after an additional 24 h, supernatants were collected as above and assayed for IL-2 activity, as above. For assessment of in vivo THF-γ2 activity, 4-week-old NTx Balb/C mice were given 14 daily intramuscular injections of 10 ng/kg of THF-γ2 each, in a total volume of 0.1 mL of PBS. Mice were sacrificed, spleen cells were exposed to Con A, and the resultant supernatants were assayed for IL-2 activity. Results are expressed as percent response (mean ± SE) of intact control mice injected with saline alone.

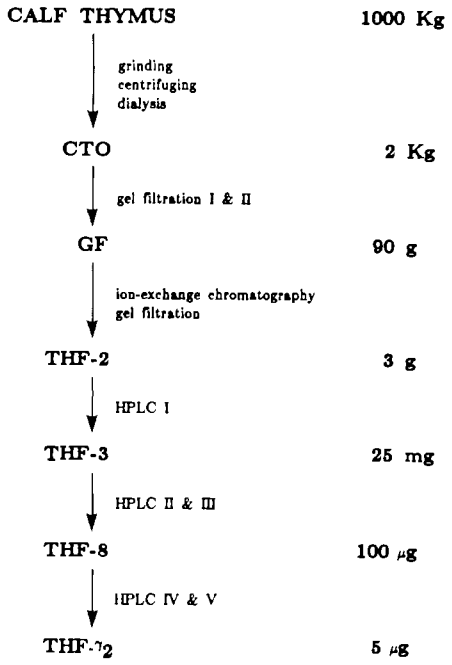

FIGURE 1: Flow diagram of the purification of THF-γ2 from calf thymus.

IL-2 Assay. IL-2 activity was assayed essentially as described by Gillis and Watson (1981). Briefly, 100-μL supernatants were added at several dilutions (1:2 to 1:32) to flat-bottomed microwells containing 10^4 cells from CTLL, an IL-2-dependent mouse cell line, in 100 μL of RPMI-1640 medium supplemented with 10% FCS (Biological Industries, Beth Haemek, Israel), 50 μM 2-mercaptoethanol, and 1 mM each of glutamine, sodium pyruvate, and nonessential amino acids. After 24 h of incubation at 37 °C in 5% CO_2/95% air, cultures were pulsed for 4 h with 1 μCi/well [^{3}H]thymidine, specific activity 5 Ci/mmol (Nuclear Research Center, Negev, Israel), and cells were harvested by using a multiple-sample automated harvester. [^{3}H]Thymidine incorporation was determined by liquid scintillation counting. Results were expressed in units per milliliter compared to a standard curve obtained with several dilutions of a semipurified IL-2 standard preparation (Life Sciences, Ness Ziona, Israel).

RESULTS

Source and Initial Isolation of THF. The scheme for isolation and purification of THF-γ2 from calf thymus is shown in Figure 1. The initial stages of the isolation procedure, from calf thymus via CTO to THF-2, were produced according to a modification of the method of Kook et al. (1975). One ton of frozen calf thymus was processed to yield ~3 g of THF-2 (0.0003%). All procedures were carried out at 4 °C, unless otherwise indicated. Briefly, THF-2 was prepared by grinding of the glands, centrifugation of the ground material, and dialysis of the supernatant. The low molecular weight dialysate, denoted CTO (calf thymus outer fraction), was further purified by sequential gel filtration through columns of Sephadex G-10 (Figure 2A) and G-25 (Figure 2B), followed by ion-exchange chromatography using a column of DEAE-Sephadex and a gradient of sodium chloride, pH 8.0 (Figure 2C). The biologically active fractions, designated THF-1, were combined, concentrated under negative pressure, and desalted by filtration through a column of Bio-Gel P-2 (data not shown). Each fraction was tested for biological activity in the in vitro PHA, Con A, MLR, and cAMP bioassays, and activity of these fractions was confirmed by in vivo reconstitution of these parameters in neonatally thymectomized mice. Fractions from the area showing bioactivity, designated THF-2, were pooled, concentrated, and used for futher purification.

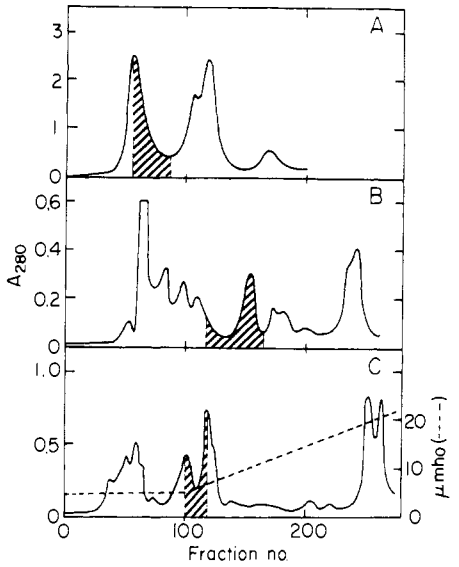

FIGURE 2: Gel filtration and ion-exchange chromatography of THF. All steps were carried out at 4 °C; hatched areas in elution profiles indicate fractions pooled and lyophilized for further purification of THF-γ2. (A) 9 g of CTO in 500 mL of water was applied to a Pharmacia K215/100 Sephadex G-10 column and eluted with water at a flow rate of 25 mL/min. The first 9 L of effluent was discarded, and then fractions of 25 mL (or 250 mL) were collected. (B) 1.5 g of the pool of THF from (A), in 60 mL of 1 mM ammonium bicarbonate, was applied to a Pharmacia K100/100 G-25 Superfine column and eluted with 1 mM ammonium bicarbonate at a flow rate of 5 mL/min. The first 2 L of the effluent was discarded, and then 50-mL fractions were collected. (C) 400 mg of the pool of THF from (B), in 50 mL of 0.1 M Tris-HCl, pH 8.0, was applied to a Pharmacia K50/100 DEAE-Sephadex A-25 column. The column was washed with 1.2 L of buffer of the same composition and then eluted with 4 L of a linear gradient of NaCl (0–0.4 M) in the same buffer, at a flow rate of 3 mL/min. Fractions of 12 mL were collected. Hatched areas show the location of in vitro THF biological activity in cAMP, PHA, Con A, and MLR assays and in vivo reconstitution of these parameters in neonatally thymectomized mice.

Purification of THF-γ2. The following procedures were performed at ambient temperature (20–24 °C). At each purification step, THF activity was monitored as a positive result of the candidate fraction in the requisite in vitro and in vivo PHA, Con A, and MLR bioassays; clinical efficacy was confirmed before proceeding to the next step (Handzel et al., 1985, 1987; Trainin et al., 1986).

Purification of THF-3. An aliquot of THF-2 containing about 3 mg of protein was applied onto a reversed-phase HPLC (C_{18}, 10 μm) column preequilibrated with 0.3 M pyridine formate at pH 4.0. The column was then eluted with a gradient of 1-propanol (0–50%) in the same buffer at a flow rate of 48 mL/h. The column eluate was monitored at 280 nm (Figure 3A). This step separated the cAMP activity from the requisite biological activities PHA, Con A, and MLR (see Figure 3A); therefore, the cAMP assay was discarded as a criterion of THF bioactivity. The biologically active fractions were combined and designated THF-3.

Purification of THF-8. THF-3 was rechromatographed under the same conditions at a flow rate of 24 mL/h using a linear gradient of 7.5–25% 1-propanol (data not shown) in pyridine formate. Aliquots of each fraction were analyzed for total amino acids after acid hydrolysis and subjected to the above bioassay procedures. Fractions having the maximum total amino acid content also showed positive results in all the requisite bioassays, PHA, Con A, and MLR. The biologically

Table I: Comparative in Vitro and in Vivo Biological Activity of THF during Successive Purification Steps

purification stage	dose	PHA	Con A	MLR
	Intact Mice[a]			
THF-3	500–5000 (ng/mL)	95 ± 50	125 ± 70	75 ± 30
THF-8	1–10 (ng/mL)	75 ± 70	110 ± 70	85 ± 40
biological THF-γ2	0.1–5 (ng/mL)	190 ± 100	130 ± 80	95 ± 50
synthetic THF-γ2	0.1–5 (ng/mL)	160 ± 70	110 ± 40	90 ± 35
	Neonatally Thymectomized Mice[b]			
THF-3	100–1000 [(ng kg^{-1} day^{-1}) × 14]	30 ± 13	51 ± 16	61 ± 24
THF-8	5–50 [(ng kg^{-1} day^{-1}) × 14]	63 ± 26	76 ± 24	62 ± 21
biological THF-γ2	1–10 [(ng kg^{-1} day^{-1}) × 14]	42 ± 19	91 ± 32	82 ± 27
synthetic THF-γ2	1–10 [(ng kg^{-1} day^{-1}) × 14]	48 ± 17	82 ± 22	79 ± 20
saline		8 ± 4	23 ± 8	29 ± 12

[a] Mouse spleen cells were incubated in microcultures with THF for 18 h and then stimulated with PHA or Con A (Rotter & Trainin, 1975) or with allogeneic irradiated spleen cells (Umiel & Trainin, 1975) for an additional 72 h. Microcultures were harvested after a 4-h pulse with [^{3}H]thymidine and counted in a β scintillation counter. Results are expressed as percent elevation (mean ± SE) of [^{3}H]thymidine incorporation relative to control cultures. [b] Four-week-old NTx mice were given 14 daily intramolecular injections of THF; spleen cells were subjected to the above PHA, Con A, and MLR bioassays. Results are expressed as percent of response (mean ± SE) of intact age-matched control mice.

active fractions were combined and concentrated to dryness under reduced pressure. The material was dissolved in water and applied to a Nucleosil C_{18} (5 μm) column, preequilibrated with 0.1% trifluoroacetic acid (TFA) at pH 2.0, and the column was eluted by using a gradient of 1-propanol in TFA. The effluent was monitored at 220 nm, and fractions of 1 mL were collected. Each fraction was analyzed as described above. The fractions containing the material eluted from the column between 16% and 22% 1-propanol had the maximum total amino acid content and biological activity. Clinical efficacy was confirmed (Handzel et al., 1985), and the fractions were designated THF-8 (Figure 3B). THF-8 was subjected to high-performance TSK gel permeation chromatography for determintion of the apparent molecular weight, which indicated that the molecular size was smaller than that of bacitracin (1500 Da) (data not shown). The THF-8 fractions were combined and recycled under the same conditions, prior to analysis of individual peptide content.

Separation of Component Peptides of THF-8. Fractions of THF-8 prepared as described above were loaded onto a column of Nucleosil C_{18} (5 μm), preequilibrated with a solution of 0.1 M $NaClO_4$, 0.1% H_3PO_4, and 22% acetonitrile, and the column was eluted with a solution of the same composition. The elution pattern was monitored at 210 nm, and fractions of 1 mL each were collected. Eight major fractions were isolated, designated as α, β, γ2, γ4, γ5, δ, ϵ, and θ, at retention times relative to a synthetic peptide which served as external standard (Figure 3C). The numbering of the γ fractions was not based on the order of elution but rather on the order of subsequent biological and chemical characterization. Portions of all the fractions other than α were digested by Pronase and proteinase K, indicating that all the fractions except α were peptides. Each fraction was dried under reduced pressure, dissolved in water, and desalted by applying them to a similar reversed-phase column which had been preequilibrated with 5% acetonitrile in TFA. The column was eluted by using a linear gradient of 5–50% acetonitrile in TFA, and the elution pattern was followed by monitoring the absorption at 210 nm. Amino acid composition analysis indicated that only the THF-γ2, -γ4, and -γ5 fractions were homogeneous. Moreover, only the THF-γ2 fraction was active in all the in vitro and in vivo PHA, Con A, and MLR bioassays; THF-γ4 and -γ5 fractions were active only in the PHA assay. The β and θ fractions showed borderline activity in the Con A assay, and the δ fraction was negative in all bioassays. Since augmentation of mitogen proliferation alone was not considered to be a criterion for specific immunological activity, these fractions were not characterized further. The comparative bioactivity of THF during successive purification steps is shown in Table I.

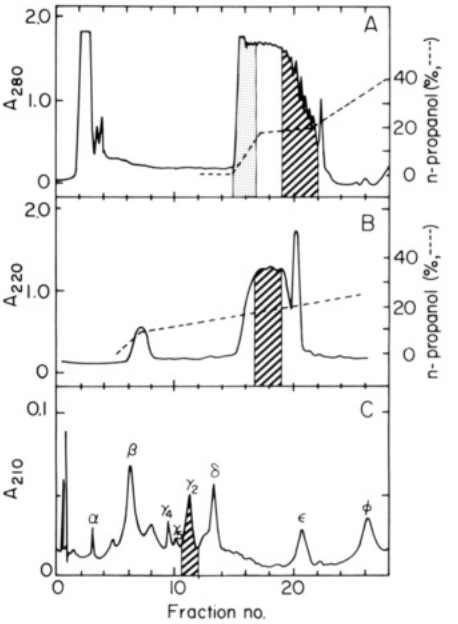

FIGURE 3: Purification of THF-γ2 from THF-2 by reversed-phase HPLC. All steps were carried out at room temperature; hatched areas in elution profiles indicate fractions pooled and dried in a SpeedVac rotary concentrator for further purification of THF-γ2. (A) 3 mg of THF-2 (see text) was applied to a Lichrosorb RP-18, 10-μm column (250 × 4 mm) and eluted with a linear gradient of 1-propanol in 0.3 M pyridine formate, pH 4.0, at a flow rate of 1 mL/min. The elution profile was monitored at 280 nm, and fractions of 2 mL were collected. The fractions containing biological activity were designated THF-3. The dotted area indicates cAMP activity. (B) Pooled fractions from rechromatographed THF-3 (see text) were loaded onto a Nucleosil-5 RP-18 (200 × 4 mm) column preequilibrated with 0.1% trifluoroacetic acid and eluted with a gradient of 1-propanol in the same solution at a flow rate of 0.4 mL/min. The elution profile was monitored at 220 nm, and fractions of 1 mL were collected. The fractons containing biological activity were designated THF-8. (C) The THF-8 pool was loaded onto a Nucleosil-5 RP-18 (200 × 4 mm) column preequilibrated with a solution of 0.1 M sodium perchlorate/0.1% phosphoric acid/22% acetonitrile and eluted isocratically with a solution of the same composition at a flow rate of 1 mL/min. The elution profile was monitored at 210 nm, and fractions of 1 mL were collected. The material from each absorption peak, α, β, γ4, γ5, γ2, δ, ϵ, and θ, was concentrated as described above and desalted on the same column (see text). Hatched areas show the location of in vitro THF biological activity in PHA, Con A, and MLR assays and in vivo reconstitution of these parameters in neonatally thymectomized mice.

Amino Acid Sequence Determination of THF-γ2. Three different preparations of purified THF-γ2, -γ4, and -γ5 were

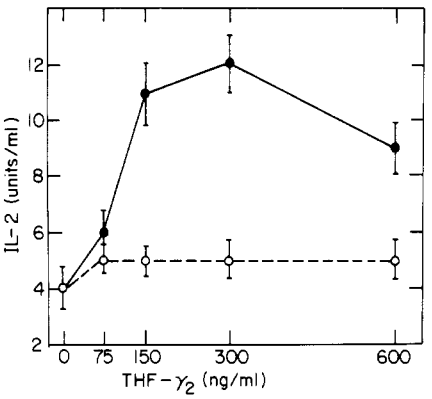

FIGURE 4: Dose–response of THF-γ2-mediated enhancement of IL-2 production. Spleen cells from (C3HeB/Fe × C57Bl/6J)F_1 mice were incubated overnight with different concentrations of THF-γ2 and then stimulated with Con A (2.5 μg/mL) for 3 h. The medium was replaced, and after an additional 21 h, supernatant samples (100 μL) were assayed for their ability to support the proliferation of CTLL, an IL-2-dependent mouse cell line (Gillis & Watson, 1981). Microcultures were harvested after a 4-h pulse with [^{3}H]thymidine. Plotted points represent the means of triplicates ± SE. Results are expressed in units of IL-2 per milliliter compared to a standard curve obtained with several dilutions of a semipurified IL-2 standard preparation. Control supernatants from Con A stimulated spleen cells preincubated with a nonrelevant peptide (Val-Ala-Gly-Pro-Leu) were included for comparison (---).

subjected to automated gas-phase amino acid sequence analyses. The sequence of THF-γ2 is Leu-Glu-Asp-Gly-Pro-Lys-Phe-Leu. The calculated molecular weight of 918 agreed with the results of the apparent molecular weight determination by gel filtration. The sequences of THF-γ4 and -γ5 were found to be His-Pro-Leu-Pro-Asp-Leu-Tyr and Phe-Val-Leu, respectively, indicating that they were not degradation products of THF-γ2.

Chemical Synthesis of THF-γ2. On the basis of the above sequence, a peptide has been chemically synthesized (to be published in a separate paper) using Merrifield's (1963) technique of solid-phase synthesis. The synthetic peptide cochromatographed with and contained all the biological activity of biological THF-γ2 (Table I).

Enhancement of IL-2 Production by THF-γ2. Figure 4 shows that in vitro pretreatment with THF-γ2 enhanced the release of IL-2 activity from Con A stimulated spleen cells of (C3HeB/Fe × C57Bl/6J)F_1 mice, low producers of IL-2. THF-γ2 caused an increase in IL-2 production in a linear dose-dependent fashion, reaching maximal stimulation at 300 ng/mL. Restoration of impaired IL-2 activity in vivo was assessed in 4-week-old NTx Balb/C mice (normal IL-2 producers) following 14 daily intramuscular injections of 10 ng/kg of THF-γ2. Spleen cells from untreated NTx mice produced negligible amounts of IL-2 when compared to spleen cells from intact age-matched normal control mice. Following treatment with THF-γ2, the level of IL-2 produced by spleen cells from NTx mice rose to 79 ± 30% (mean ± SE) of that observed in the controls.

DISCUSSION

We have purified to homogeneity an octapeptide, THF-γ2, which retains essentially all of the biological activity of the crude hormone from which it derived. THF-γ2 has a calculated molecular weight of 918 and has the following amino acid sequence: Leu-Glu-Asp-Gly-Pro-Lys-Phe-Leu. Although the molecular size is similar to that of the nonapeptide serum thymic factor (FTS) (Bach et al., 1977), the sequence of THF-γ2 showed no homology to the published sequence of FTS or any other thymic hormone nor is it identical with part of any peptide or protein of known sequence. The purification of a specific peptide with several immunoregulatory capacities affirms our previous assumption that a single peptide hormone was responsible for the major biological activities of crude THF preparations.

During the last decade, there have been many reports concerning purification and characterization of various thymic hormones. The search for active principals has been conducted either on the basis of random chemical sorting, which presents not only the theoretical advantage of screening a large number of defined peptides but also the risk of obtaining biologically irrelevant materials, or on the basis of well-defined, time-consuming bioassays. Progress in the purification of THF-γ2 has been slow because it was purified by using the latter approach, limiting the choice of chromatographic conditions to those that would not destroy bioactivity. Moreover, solvents employed had to be nontoxic when injected into humans because fractions at each step of purification were submitted for confirmation of clinical efficacy (Trainin et al., 1985; Handzel et al., 1985, 1987). In addition, the development of the procedure was hampered through the THF-8 step by lack of a distinct peak which could be monitored either by fluorescamine detection or by UV absorption. Therefore, the process was monitored solely by amino acid composition analysis and by a battery of in vitro bioassays and in vivo immunological reconstitution procedures on each fraction.

A major problem encountered during large-scale production of THF-8 to be used for chemical characterization was the great variability among batches of crude THF with regard to content of the THF-γ2 peak. Since thymuses obtained for production of the starting material were frozen, it is likely that often the peptide was at least partially degraded during the isolation procedure. It remains possible that a precursor molecule with a molecular weight higher than 900 may exist because protease inhibitors were omitted from the purification scheme reported here. The yields were very low and varied somewhat from batch to batch; on the average, 1 ton of wet thymus yielded between 0.5 and 10 μg of THF-γ2. The "specific activity" could not be calculated because the bioassays for THF could at best be considered only semiquantitative. Nevertheless, the dosage required for optimal activity decreased by 1 or 2 orders of magnitude with each purification step.

THF-γ2 treatment augmented in vitro IL-2 production in spleen cells obtained from a low-IL-2-producing mouse strain and restored deficient in vivo IL-2 production by spleen cells from NTx mice. Similar results were obtained in human peripheral blood lymphocytes obtained from patients after in vivo THF-γ2 treatment for immune impairments (data not shown). In a recent study, several other well-defined thymic preparations, including thymosin fraction 5 (TF5), thymostimulin, thymic factor X, porcine TF5, leucotrofina, thymopentin, and thymulin, were compared for their effect on IL-2 production by human peripheral blood lymphocytes (Zatz et al., 1985). Of these, only TF5 was able to enhance IL-2 production, but the biological activity was not due to the presence of thymosins α_1 or β_4. Thus, it is possible that one of the unidentified components of TF5 preparations might be THF-γ2, which could have been responsible for the observed effect on IL-2 production.

THF-γ2 augmented multiple T-cell functions and stimulated production of a lymphokine; thus, it may be of more value than single agents for immunotherapy of immune deficiency states. The availability of a purified molecule will facilitate the investigation of regulation of biosynthesis of the hormone as well

as the study of structure–function relationships.

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Peptides Mimicking the Flap of Human Renin: Synthesis, Conformation, and Antibody Recognition†

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ABSTRACT: Four peptides related to human renin flap region have been synthesized. Two of them are ring closed through appropriately designed disulfide bridges. Structure analysis involving IR and NMR techniques and recognition by polyclonal human renin antibodies provides support for a β-hairpin secondary structure of the cyclized peptides identical with that presented by the flap section in the speculative human renin model [Blundell, T., Sibanda, B. L., & Pearl, L. (1983) *Nature (London) 304*, 273–275; Sibanda, B. L., Blundell, T., Hobart, P. M., Fogliano, M., Bindra, J. S., Dominy, B. W., & Chirgwin, J. M. (1984) *FEBS Lett. 174*, 102–111].

Renin (EC 3.4.23.15), an aspartyl protease, plays a key role in the regulation of blood pressure homeostasis. It cleaves its substrate angiotensinogen to release angiotensin I which is then converted into angiotensin II, a potent vasoconstrictor octapeptide, by the action of a carboxydipeptidase. Apart from its primary structure which has been recently established from the sequence of its cDNA (Imai et al., 1983), no experimental data have been obtained concerning the structure of renins. Several renin three-dimensional models have been proposed (Blundell et al., 1983; Sibanda et al., 1984; Akahane et al., 1985; Carlson et al., 1985) on the basis of X-ray diffraction data which show that, except for small deviations, there is a striking similarity with the structures of pepsins (Tang et al., 1978). These models can be used as a working hypothesis in order to predict, at least in part, the conformation of renins.

The aspartyl proteases are characterized by a major groove between the C- and N-terminal domains and in addition by

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