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Effects of Synthetic Thymopoietin Fragments on Low E-Rosette Forming Cells in a Patient with Lupus Nephritis[1)]

Takashi Abiko* and Hiroshi Sekino

Kidney Center, Sendai Insurance Hospital, Tsutsumimachi 3-16-1, Sendai, 980, Japan

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Two thymopoietin fragments, H–Val–Thr–Leu–Pro–Ala–Gly–Glu–Gln–Arg–Lys–Asp–Val–Tyr–OH (positions 24—36) and H–Glu–Gln–Arg–Lys–Asp–Val–Tyr–OH (positions 30—36), were synthesized using protecting groups removable by hydrogen fluoride treatment. The *in vitro* additions of these synthetic fragments could restore the low E-rosette forming capacity of cells in a patient with lupus nephritis to normal levels. The *in vitro* effects of tridecapeptide (positions 24—36) and heptapeptide (positions 30—36) fragments of thymopoietin on the low E-rosette forming capacity of cells in a patient with lupus nephritis were also compared with that of the synthetic pentapeptide fragment (positions 32—36). The relative potency of the heptapeptide was 65.22 and that of the tridecapeptide was 40.54 based on the pentapeptide (100.00) as a standard.

Keywords——thymopoietin; lupus nephritis; low E-rosette forming cells; HOBT–DCC procedure; anisole–thioanisole–*o*-cresol; defect of cell-mediated immunity

Thymopoietin is a polypeptide hormone of the thymus that consists of a 49 amino acid polypeptide chain.[2,3)] In 1977, Fujino *et al.*[4)] reported the first total synthesis of thymopoietin II using the HONB–DCC procedure;[5)] the synthetic peptide was active in the induction of thymocyte differentiation from prothymocytes *in vitro* and the potency of this synthetic thymopoietin was more than 10 times the potency of the synthetic tridecapeptide corresponding to the sequence 29—41 of the hormone.[6)] Recently, Goldstein *et al.*[7)] reported the synthesis of a pentapeptide corresponding to amino acids 32 to 36 of thymopoietin, and showed that it also has biological properties characteristic of thymopoietin. Then, we reported that the decapeptide (positions 32—41)[8)] and the pentapeptide (positions 32—36)[9)] induce some recovery of E-rosette formation in the uremic state.

On the other hand, it is generally accepted that a high percentage of lupus nephritis patients have a defect of cell-mediated immunity.[10,11)] A decrease of E-rosette forming cells in these patients has been demonstrated by several investigators.[12)] We described here the synthesis of two thymopoietin fragments corresponding to positions 30—36 (H–Glu–Gln–Arg–Lys–Asp–Val–Tyr–OH) and 24—36 (H–Val–Thr–Leu–Pro–Ala–Gly–Glu–Gln–Arg–Lys–Asp–Val–Tyr–OH). Further, we compared the *in vitro* effects of these two peptide fragments and thymopoietin pentapeptide (positions 32—36)[9)] on low E-rosette forming cells in a patient with lupus nephritis. The relative responses of the low E-rosette forming cells of a lupus nephritis patient to the heptapeptide (positions 30—36) and the tridecapeptide (positions 24—36) were compared with that to the pentapeptide (positions 32—36) by taking synthetic pentapeptide[9)] as a standard.

In the present synthesis, protecting groups of amino acid derivatives, Z–Val, Arg(NO_2), Asp(OBzl), Glu(OBzl), Lys(Z) and Tyr(OBzl), were removed by hydrogen fluoride treatment.[13)] These protecting groups survive mostly intact under careful TFA–anisole treatment for the removal of the Boc group, employed as a temporary α-amino protecting group. The synthetic route to the C-terminal heptapeptide, H–Glu–Gln–Arg–Lys–Asp–Val–Tyr–OH (III), is illustrated in Chart 1. The protected heptapetide ester, Boc–Glu(OBzl)–Gln–Arg(NO_2)–Lys(Z)–Asp(OBzl)–Val–Tyr–OBzl (II) was first prepared stepwise by the Np method[14)] and then by the HOBT–DCC procedure[15)] starting from Boc–Arg(NO_2)–Lys(Z)–Asp(OBzl)–Val–

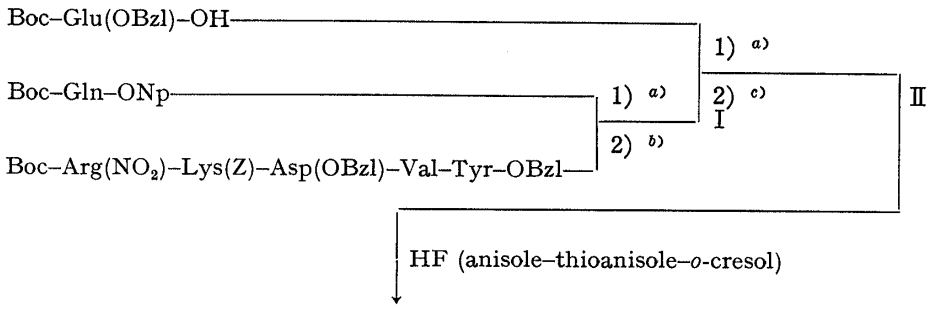

Chart 1. Synthetic Scheme for the Heptapeptide (positions 30—36)

a) TFA-anisole. *b*) Active ester. *c*) HOBT-WSCI.

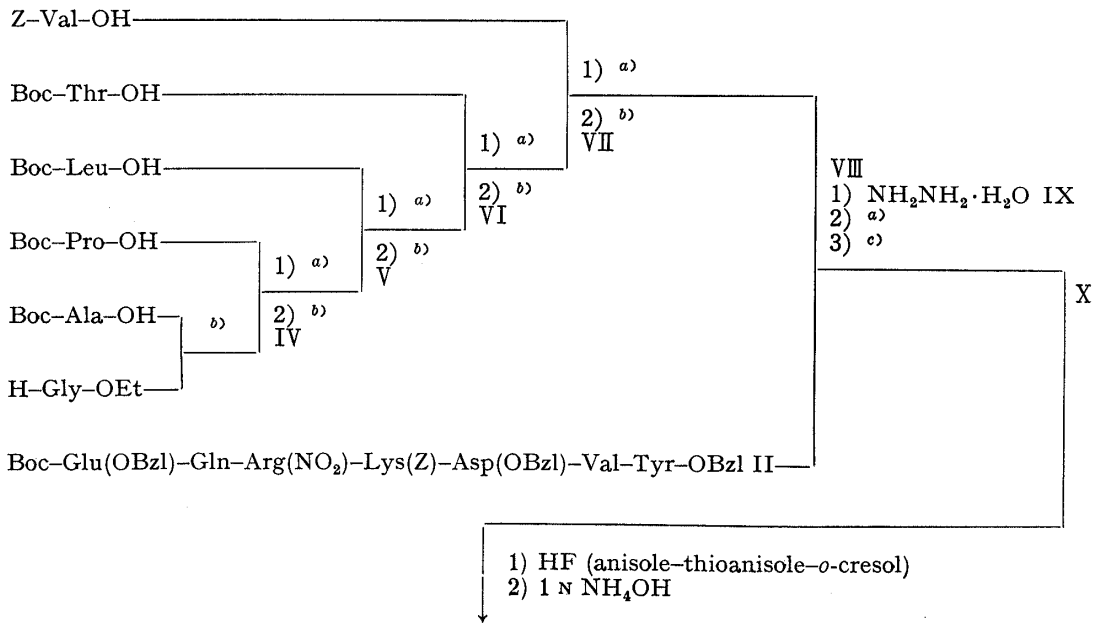

Chart 2. Synthetic Scheme for the Tridecapeptide (positions 24—36)

a) TFA-anisole. *b*) HOBT-WSCI. *c*) Azide.

Tyr–OBzl.[9] The protected heptapeptide II was treated with hydrogen fluoride, in the presence of anisole–thioanisole–*o*-cresol (1 : 1 : 1, v/v)[16] to suppress a possible side reaction at the Tyr residue,[17] to remove all protecting groups. The deprotected peptide was converted to the corresponding acetate by treatment with Amberlite CG-4B (acetate form) and purified by gel-filtration on Sephadex G-25. The absorbancy at 275 nm due to the Tyr residue was used as a marker during this purification. The homogeneity of the synthetic heptapeptide, H–Glu–Gln–Arg–Lys–Asp–Val–Tyr–OH (III), thus obtained was assessed by paper chromatography in two different solvent systems. The amino acid compositions in the 4 N MSA hydrolysate and aminopeptidase (AP–M)[18] digest of III agreed well with the theoretical values. The synthetic route to the N-terminal hexapeptide hydrazide, Z–Val–Thr–Leu–Pro–Ala–Gly–$NHNH_2$ (IX), is illustrated in Chart 2. In order to synthesize this peptide hydrazide (IX), the N-terminal protected hexapeptide ester, Z–Val–Thr–Leu–Pro–Ala–Gly–OEt (VIII), was first prepared stepwise by the HOBT–DCC procedure[15] starting from H–Gly–OEt·HCl. This protected hexapeptide ester (VIII) was converted to the corresponding hydrazide, Z–Val–

Thr–Leu–Pro–Ala–Gly–$NHNH_2$ (IX), by the usual hydrazine treatment, which permitted us to check its homogeneity by elemental analysis. The protected heptapeptide ester II was converted to the corresponding amine by TFA–anisole treatment and was subjected to coupling with the azide derived from IX by treatment with HCl, followed by isoamylnitrite, to afford the protected tridecapeptide ester, Z–Val–Thr–Leu–Pro–Ala–Gly–Glu(OBzl)–Gln–Arg(NO_2)–Lys(Z)–Asp(OBzl)–Val–Tyr–OBzl (X), which was purified by column chromatography on silica gel. Its homogeneity was confirmed by elemental analysis and amino acid analysis of the 4 N MSA hydrolysate. The protected tridecapeptide ester thus obtained was treated with hydrogen fluoride, in the presence of anisole–thioanisole–*o*-cresol (1 : 1 : 1, v/v)[16] to suppress side reaction of the Tyr residue, [17] to remove all protecting groups. The deblocked peptide was precipitated by dry ether, converted to the corresponding acetate by treatment with Amberlite CG-4B (acetate form) and then treated with 1 N NH_4OH for 30 min. The latter treatment was performed because of the reversible N→O shift at the Thr residue during the hydrogen fluoride treatment.[18,19] Finally, the product was purified by gel-filtration on Sephadex G-15 using 1% AcOH, followed by partition column chromatography on Sephadex G-25 according to Yamashiro.[20] The absorbancy at 275 nm due to the Tyr residue was used as a marker during these purification processes. The homogeneity of the tridecapeptide (XI) thus obtained was assessed by paper chromatography in two different solvent systems and determination of the amino acid composition in the 4 N MSA hydrolysate. Despite the presence of the Pro residue,[21] complete digestion of this synthetic peptide with commercial AP–M[22,23] was achieved and the presence of a Gln residue in the product was thus confirmed . The *in vitro* effects of the synthetic thymopoietin fragments on E-rosette forming cells in a lupus nephritis patient are shown in Table I.

Table I. Effects of the Synthetic Thymopoietin Fragments on the Low E-Rosette Forming Cells in a Patient with Lupus Nephritis

Peptide	Dose (molar concentration)	E-Rosette forming cells (%)
—*a*)		74±5
—*b*)		46±6
Pentapeptide*b,c*) (positions 32—36)	1.5×10^{-4}	70±5
Heptapeptide*b,c*) (positions 30—36)	2.3×10^{-4}	71±6
Tridecapeptide*b,c*) (positions 24—36)	3.7×10^{-4}	68±6

a) Normal lymphocytes.
b) Patient's lymphocytes.
c) Incubation was carried out for 1 h at 37°C with synthetic peptide.

Incubation of blood from a lupus nephritis patient in the presence of various amounts of synthetic fragment peptides from 1.5×10^{-4} to 3.7×10^{-4} M resulted in recovery of E-rosette formation (Table I). The relative potency of the synthetic heptapetptide (positions 30—36) was 65.22 and that of the tridecapeptide (positions 24—36) was 40.54 based on the pentapeptide (positions 32—36)[9] (100.00) as a standard. These results strongly suggest that the shorter chain peptide containing the active site (positions 32—36) of thymopoietin possesses much higher *in vitro* ability to increase the activity of E-rosette forming cells than the longer chain peptide in cases of lupus nephritis.

Experimental

Melting points are uncorrected. Rotations were measured with an Atago Polax machine (cell length: 10 cm). Amino acid compositions of 4 N MSA hydrolysates and AP-M digests were determined with a JEOL JLC-8AH amino acid analyzer (one-column system). Concentration of solutions was carried out in a rotary

evaporator under reduced pressure at 35 to 40°C. Boc groups of the protected peptides were removed by TFA–anisole treatment. The resulting amino components were chromatographed on filter paper, Toyo Roshi No. 51, at room temperature. Rf^1 values refer to the Partridge system[24] and Rf^2 values refer to BuOH–pyridine–AcOH–H_2O (30: 20: 6: 24).[25] Venous blood from a lupus nephritis patient and normal subjects was drawn into heparinized syringes and sedimented at room temperature. Aminopeptidase (3501, Aminopeptidase 210520) was purchased from the Protein Research Foundation, Osaka, Japan. Methanesulfonic acid containing 0.2% 3-(2-aminoethyl)indole (Lot No. 12040) was purchased from Techno Chemical Co.

Boc-Gln-Arg(NO_2)-Lys(Z)-Asp(OBzl)-Val-Tyr-OBzl (I)——Boc–Arg(NO_2)–Lys(Z)–Asp(OBzl)–Val–Tyr–OBzl[9] (570 mg) was treated with TFA (3 ml)-anisole (0.3 ml) at room temperature for 30 min, then dry ether was added. The resulting powder was collected by filtration, dried over KOH pellets *in vacuo* and then dissolved in DMF (5 ml). Boc–Gln–ONp (325 mg) was added to the above solution, followed by NMM[26] to keep the solution slightly alkaline. After 24 h at room temperature, the reaction mixture was diluted with 1 N NH_4OH (1 ml) with stirring to saponify the unchanged *p*-nitrophenyl ester. After 1 h, the mixture was poured into ice-chilled 1 N NH_4OH with stirring. Then, 50% NH_4OAc was added dropwise with stirring to form a precipitate. The precipitate was collected and washed successively with 1 N NH_4OH, H_2O, 1 N citric acid and H_2O. The product was recrystallized from MeOH and ether; yield 439 mg (65%), mp 150—167°C, $[\alpha]_D^{26}$ −41.3° (c=1.0, DMF), Rf^1 0.79, Rf^2 0.86, single ninhydrin-positive spot. *Anal.* Calcd for $C_{62}H_{82}N_{12}O_{17}\cdot 2H_2O$: C, 57.13; H, 6.65; N, 12.90. Found: C, 56.86; H, 6.47; N, 12.70.

Boc-Glu(OBzl)-Gln-Arg(NO_2)-Lys(Z)-Asp(OBzl)-Val-Tyr-OBzl (II)——I (127 mg) was treated with TFA (1 ml)–anisole (0.1 ml) at room temperature for 30 min, then excess TFA was removed by evaporation. The residue was washed with dry ether and dried over KOH pellets *in vacuo* and the deprotected peptide was dissolved in DMF (3 ml). To this ice-chilled solution, NMM (0.01 ml), Boc–Glu(OBzl)–OH (40 mg), HOBT (15 mg) and WSCI (18 mg) were successively added. After stirring overnight at 0°C, the mixture was poured into ice-chilled 1 N $NaHCO_3$ with stirring. Then, 50% NH_4OAc was added dropwise with stirring to form a precipitate. The precipitate was collected and washed successively with 1 N $NaHCO_3$, H_2O, 1 N citric acid and H_2O. The product was reprecipitated from MeOH and ether; yield 121 mg (81%), mp 155—164°C, $[\alpha]_D^{29}$ −25.3° (c=1.0, DMF), Rf^1 0.87, Rf^2 0.91, single ninhydrin-positive spot. *Anal.* Calcd for $C_{74}H_{95}N_{13}O_{20}$: C, 59.78; H, 6.44; N, 12.25. Found: C, 59.53; H, 6.31; N, 11.89.

H-Glu-Gln-Arg-Lys-Asp-Val-Tyr-OH (III)——The protected heptapeptide II (120 mg) was treated with HF (approximately 3 ml) in the presence of anisole–thioanisole–*o*-cresol (1: 1: 1 v/v, 0.5 ml) in an ice-bath for 1 h. After removal of the HF, dry ether was added and the resulting powder was dissolved in H_2O (5 ml). The solution was treated with Amberlite CG-4B (acetate form, approximately 1 g) for 30 min, filtered by suction, and evaporated to dryness *in vacuo*. The crude peptide thus obtained was dissolved in 1% AcOH (2 ml), applied to a column of Sephadex G-15 (2.8×90 cm), and eluted with the same solvent. Ultraviolet absorption at 275 nm was measured in each fraction (4 ml). The fractions corresponding to the main peak (tube Nos. 81—90) were combined and the solvent was removed by lyophilization to give a fluffy powder; yield 39 mg (51%), mp 211—219°C (dec.), $[\alpha]_D^{23}$ −51.8° (c=1.0, 1 N AcOH), Rf^1 0.06, Rf^2 0.11, single ninhydrin- and Sakaguchi-positive spot. Amino acid ratios in 4 N MSA hydrolysate: Arg 0.83, Lys 0.86, Tyr 0.91, Val 1.00, Asp 0.79, Glu 1.78 (recovery of Val 81.2%). Amino acid ratios in AP-M digest: Arg 1.01, Lys 0.92, Tyr 1.03, Val 1.00, Glu 0.87, Gln 0.84, Asp 0.85 (recovery of Val 80.3%).

Boc-Ala-Gly-OEt (IV)——HOBT (1.6 g) and WSCI (1.8 g) were added to a solution of Boc–Ala–OH (2.1 g), H–Gly–OEt·HCl (1.4 g) and NMM (1.1 ml) in DMF (10 ml) with stirring at 0°C. The reaction mixture was stirred for 12 h at 4°. Then, the reaction mixture was extracted with EtOAc and the extract was washed successively with 1 N citric acid, H_2O, 1 N $NaHCO_3$ and H_2O, dried over $MgSO_4$ and then concentrated *in vacuo*. The residue was reprecipitated from EtOAc and *n*-hexane; yield 1.7 g (oily material) (61%), $[\alpha]_D^{21}$ −19.4° (c=1.0, DMF), Rf^1 0.72, Rf^2 0.81, single ninhydrin-positive spot. *Anal.* Calcd for $C_{12}H_{22}N_2O_5$: C, 52.54; H, 8.08; N, 10.21. Found: C, 52.27; H, 7.93; N, 10.01.

Boc-Pro-Ala-Gly-OEt (V)——IV (1.4 g) was treated with TFA (5 ml)–anisole (0.5 ml) as described above. To ice-chilled solution of the resulting dipeptide ester trifluoroacetate in DMF (10 ml), Boc–Pro–OH (1.3 g), HOBT (744 mg) and WSCI (854 mg) were added, followed by addition of NMM (0.6 ml) to keep the solution slightly alkaline. After 12 h at 0°C, the reaction mixture was extracted with EtOAc and the extract was washed successively with 1 N citric acid, H_2O, 1 N $NaHCO_3$ and H_2O, and dried over $MgSO_4$. Next, the solution was evaporated to dryness *in vacuo* and the residue was reprecipitated from EtOAc and *n*-hexane; yield 1.3 g (65%), mp 56—58°C, $[\alpha]_D^{26}$ −16.4° (c=1.0, DMF), Rf^1 0.64, Rf^2 0.66, single ninhydrin-positive spot. *Anal.* Calcd for $C_{17}H_{29}N_3O_6\cdot H_2O$: C, 52.43; H, 8.02; N, 10.79. Found: C, 52.36; H, 7.82; N, 10.59.

Boc-Leu-Pro-Ala-Gly-OEt (VI)——This compound was prepared from V (619 mg), Boc–Leu–OH (458 mg), HOBT (248 mg) and WSCI (285 mg) essentially as described for the preparation of IV. The product was reprecipitated from tetrahydrofuran and petroleum ether; yield 541 mg (67%), mp 64—68°C, $[\alpha]_D^{26}$ −31.0° (c=1.0, DMF), Rf^1 0.81, Rf^2 0.84, single ninhydrin-positive spot. *Anal.* Calcd for $C_{23}H_{40}N_4O_7$: C, 57.00; H, 8.32; N, 11.56. Found: C, 56.72; H, 7.94; N, 11.73.

Boc-Thr-Leu-Pro-Ala-Gly-OEt (VII)——This compound was prepared from VI (606 mg), Boc–Thr–OH

(301 mg), HOBT (186 mg) and WSCI (213 mg) essentially as described for the preparation of V; yield 468 mg (62%), mp 64—71°C, $[\alpha]_D^{26}$ −26.8° (c=1.0, DMF), Rf^1 0.88, Rf^2 0.90, single ninhydrin-positive spot. *Anal.* Calcd for $C_{27}H_{47}N_5O_9 \cdot H_2O$: C, 53.71; H, 8.18; N, 11.60. Found: C, 53.50; H, 8.12; N, 11.34.

Z-Val-Thr-Leu-Pro-Ala-Gly-OEt (VIII)——This compound was prepared from VII (418 mg), Z–Val–OH (197 mg), HOBT (107 mg) and WSCI (122 mg) essentially as described for the preparation of V. The reaction mixture was extracted with EtOAc and the extract was washed successively with 1 N HCl, H_2O, 1 N $NaHCO_3$ and H_2O, dried over $MgSO_4$ and concentrated *in vacuo*. The residue was precipitated from EtOAc and petroleum ether; yield 328 mg (64%), mp 89—103°C, $[\alpha]_D^{26}$ −31.4° (c=1.0, DMF). For paper chromatography, the Z group of VIII was removed by catalytic hydrogenation in the usual manner: Rf^1 0.76, Rf^2 0.81, single ninhydrin-positive spot. *Anal.* Calcd for $C_{35}H_{54}N_6O_{10}$: C, 58.48; H, 7.57; N, 11.69. Found: C, 58.28; H, 7.52; N, 11.55.

Z-Val-Thr-Leu-Pro-Ala-Gly-NHNH$_2$ (IX)——VIII (205 mg) was dissolved in MeOH (3 ml). To this solution, hydrazine hydrate (0.28 ml) was added and the mixture was left to stand at room temperature for 48 h. After removal of the MeOH by evaporation, the residue was recrystallized from MeOH and ether; yield 158 mg (79%), mp 190—195°C, $[\alpha]_D^{26}$ −71.8° (c=1.0, DMF). *Anal.* Calcd for $C_{33}H_{52}N_8O_9$: C, 56.23; H, 7.44; N, 15.90. Found: C, 56.41; H, 7.74; N, 15.98.

Z-Val-Thr-Leu-Pro-Ala-Gly-Glu(OBzl)-Gln-Arg(NO$_2$)-Lys(Z)-Asp(OBzl)-Val-Try-OBzl (X)——II (149 mg) was treated with TFA (1 ml)–anisole (0.1 ml) as usual and dry ether was added. The resulting powder was collected by filtration, dried over KOH pellets *in vacuo* and dissolved in DMF (2 ml) containing NMM (0.01 ml). The azide[27] (prepared from 92 mg of Z–Val–Thr–Leu–Pro–Ala–Gly–$NHNH_2$ with 0.03 ml of 6 N HCl in dioxane and 0.03 ml of isoamylenitrite at −60°C) in DMF (2 ml) and NMM (0.05 ml) were added to the above ice-chilled solution and the mixture was stirred for 48 h at 4°C. Then the mixture was poured into ice-chilled 1 N $NaHCO_3$ with stirring. Next, 50% NH_4OAc was added dropwise with stirring to form a precipitate. The precipitate was collected and washed successively with 1 N $NaHCO_3$, H_2O, 1 N HCl and H_2O. The product was further purified by column chromatography on silica gel (1.8×60 cm), equilibrated and eluted with $CHCl_3$–water-saturated BuOH (1: 1). The desired fractions (4 ml each, tube Nos. 28—32) were combined and the solvent was removed by evaporation. Ether was added to the residue to give a precipitate. The product was recrystallized from hot EtOAc; yield 158 mg (76%), mp 110—119°C $[\alpha]_D^{26}$ −33.4° (c=1.0, DMF). Amino acid ratios in 4 N MSA hydrolysate: Lys 0.84, Thr 0.82, Pro 0.81, Leu 1.03, Val 2.14, Gly 1.00, Arg(NO_2) 0.89, Glu 1.84, Ala 1.12, Asp 0.90, Tyr 0.88 (recovery of Gly 82.4%). *Anal.* Calcd for $C_{102}H_{128}N_{19}O_{28}$: C, 59.23; H, 6.24; N, 12.87. Found: C, 58.87; H, 6.37; N, 12.44.

H-Val-Thr-Leu-Pro-Ala-Gly-Glu-Gln-Arg-Lys-Asp-Val-Tyr-OH (XI)——The protected tridecapeptide X (100 mg) was treated with HF (approximately 3 ml) in the presence of anisole–thioanisole–*o*-cresol (1: 1: 1 v/v, 0.5 ml) in ice-bath for 1 h. After removal of the HF, dry ether was added and the resulting powder was dissolved in H_2O (5 ml). The solution was treated with Amberlite CG-4B (acetate form, approximately 2 g) for 30 min, and filtered by suction. The filtrate was evaporated to dryness *in vacuo*, then 1 N NH_4OH (2 ml) was added to the residue. The solution was left to stand at 0°C for 30 min, and lyophilized. The crude peptide was dissolved in 1% AcOH (2 ml), applied to a column of Sephadex G-15 (2.8×96 cm), and eluted with the same solvent. Fractions of 5 ml were collected per 16 min, and the absorption at 275 nm was determined. Fractions corresponding to the front main peak (tube Nos. 53—61) were combined and the solvent was removed by lyophilization. The fluffy powder thus obtained was dissolved in small amount of the upper phase of a solvent system consisting of BuOH–AcOH–H_2O (4: 1: 5, by volume). The solution was subjected to partition column chromatography on Sephadex G-25 (2.8×98 cm) previously equilibrated with the lower phase of the above solvent system. The column was developed with the same upper phase, fractions of 4 ml were collected (one fraction per 25 min) and the absorbancy at 275 nm was determined. The fractions corresponding to the main peak (tube Nos. 58—65) were combined and evaporated to dryness *in vacuo*, and the residue was lyophilized to give a fluffy materials; yield 31 mg (44%), mp 198—209°C (dec.), $[\alpha]_D^{23}$ −63.7° (c=1.0, 1 N AcOH), Rf^1 0.05, Rf^2 0.14, single ninhydrin- and Sakaguchi-positive spot. Amino acid ratios in 4 N MSA hydrolysate: Arg 0.83, Lys 0.92, Leu 1.13, Ala 1.12, Val 2.03, Pro 0.81, Gly 1.00, Tyr 0.93, Asp 0.87, Glu 1.74, Thr 0.79 (recovery of Gly 84.3%). Amino acid ratios in AP-M digest: Arg 0.90, Lys 0.82, Leu 0.97, Ala 1.12, Val 2.06, Pro 0.85, Gly 1.00, Tyr 0.88, Asp 0.91, Glu 1.06, Gln+Thr 1.77 (Calcd as Thr) (recovery of Gly 78.1%).

E-Rosette Formation——A patient's blood was incubated with the synthetic peptide for 1 h at 37°C and then lymphocytes were isolated in a Hypaque–Ficoll gradient[28] for testing of E-rosette formation. Isolated lymphocytes were adjusted to 5×10^5 cells/ml with PBS. Contamination by monocytes and polymorphonuclear cells amounted to less than 7%.[29] Sheep erythrocytes (Kyokutō Pharmaceutical Co.) were washed with PBS, and a suspension (8×10^6/ml) was prepared. The lymphocytes were washed with GVB^{2+} and centrifuged for 10 min at 1500 rpm, then suspended in FCS (Dainippon-Pharmaceutical Co.) (1 ml). The suspension was mixed with the suspension of sheep erythrocytes (0.5 ml) and incubated for 16 h at 4°C. The mixture was then centrifuged for 5 min at 900 rpm. Triplicate wet-cell preparations were checked by phase contrast microscopy. For each preparation, 200 lymphocytes were counted, and the proportion binding more than three erythrocytes was determined.

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References and Notes

1) The amino acid residues except glycine are of the L-configuration. The abbreviations used to denote amino acid derivatives and peptides are those recommended by the IUPAC-IUB Commission on Biochemical Nomenclature: *Biochemistry*, **11**, 1726 (1972). Other abbreviations: Z=benzyloxycarbonyl, OBzl=benzyl ester, ONp=*p*-nitrophenyl ester, Boc=*t*-butoxycarbonyl, OEt=ethyl ester, DMF=dimethylformamide, TFA=trifluoroacetic acid, WSCI=1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide, DCC=dicyclohexylcarbodiimide, HOBT=N-hydroxybenzotriazole, HONB=N-hydroxy-5-norbornene-2,3-dicarboximide, MSA=methanesulfonic acid, AcOH=acetic acid, EtOAc=ethyl acetate, HF=hydrogen fluoride, NMM=N-methylmorpholine, FCS=fetal calf serum, GVB^{2+}=gelatin veronal buffer, PBS=phosphate-buffered saline, E=sheep erythrocytes.
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