tion in the blood. Because the formation of MTP is a second-order reaction, the apparent reaction rate is affected by the concentrations of both hydralazine and pyruvic acid. Thus, it is necessary to study the reaction with drug levels of the same order as that in blood.

The Effect of Thymosin \( \alpha_1 \) Fragments on T-Lymphocyte Transformation in the Uremic State

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The thymosin \( \alpha_1 \), C-terminal fragment H-Lys-Asp-Leu-Lys-Glu-Lys-Lys-Glu-Val-Val-Glu-Glu-Ala-Glu-Asn-OH (positions 14—28) was synthesized by a conventional method. Two thymosin \( \alpha_1 \) fragments, H-Lys-Lys-Glu-Val-Val-Glu-Ala-Glu-Asn-OH (positions 19—28) and the pentadecapeptide fragment synthesized in this study, were tested for effect on lymphocyte transformation in the uremic state. The synthetic pentadecapeptide increased \(^3\)H-thymidine incorporation into DNA in the uremic state, but the synthetic decapptide had no effect on the \(^3\)H-thymidine incorporation.

Keywords—thymosin \( \alpha_1 \) fragment; uremic serum; chronic renal failure; HONB-DCC; lymphocytes transformation

1) Abbreviations used are those recommended by the IUPAC-IUB Commission on Biochemical Nomenclature: Biochemistry, 11, 1726 (1972). Other abbreviations: DMF, dimethylformamide; PHA, phytohemagglutinin; WSCI, water-soluble carbodiimide; MA, mixed anhydride; TFA, trifluoroacetic acid; HONB, N-hydroxy-5-norbornene-2,3-dicarboximide; HOBT, N-hydroxybenzotriazole; DCC, dicyclohexylcarbodiimide.

2) Location: a) Tsutsumimachi 3-16-1, Sendai, 980, Japan; b) Higashi-shichibanchō 84, Sendai, 980, Japan.
Goldstein et al. prepared a biologically active heat-stable polypeptide, named thymosin, from bavine thymus. Thymosin \( \alpha_1 \), an immunologically active polypeptide, highly acidic with an isoelectric point of 4.2. This molecule is composed of 28 amino acid residues with acetylserine as the NH\(_2\) terminus. Thymosin \( \alpha_1 \) is a potent immunopotentiating agent. It is from 10 to 1000 times as active as the parent thymosin fraction 5 preparation in a number of bioassay systems designed to measure the maturation and function of T-lymphocytes. Partially purified bovine thymosin preparations, when administered to neonatally thymectomyimized mice, have been shown to slow the development of wasting disease, and to increase the incidence of cell-mediated immune responses.

On the other hand, the transformation of the T-lymphocytes into lymphoblasts with mitotic activity after antigen stimulation is known to be depressed in chronic renal failure. The resulting loss of cellular immunity is demonstrable by measurement of \(^3\)H-thymidine uptake in lymphocyte cultures after PHA stimulation. Addition of uremic serum to the cultures decreases metabolic DNA synthesis as well as impairing cell viability. The authors have synthesized a C-terminal thymosin \( \alpha_1 \) fragment (positions 14—28) by a conventional method in order to examine its effect on lymphocyte transformation in the uremic state. The synthesis of the C-terminal decapetide (positions 19—28) was described in the preceding paper, and the synthetic route to the pentadecapeptide (positions 14—28) is illustrated in Fig. 1.

![Synthetic Scheme](image-url)

Fig. 1. Synthetic Scheme for the Thymosin \( \alpha_1 \) Fragment (Residues 14—18)

Boc–Lys(Z)–Lys(Z)–Glu(OBzl)–Val–Val–Glu(OBzl)–Glu(OBzl)–Ala–Glu(OBzl)–Asn–ONb was treated with TFA in the presence of anisole to remove the Boc group and the resulting product was condensed with Boc–Glu(OBzl)–OH by the HOBT–DCC procedure to give Boc–Glu(OBzl)–Lys(Z)–Lys(Z)–Glu(OBzl)–Val–Val–Glu(OBzl)–Glu(OBzl)–Ala–Glu(OBzl)–Asn–ONb (I). H–Lys(Z)–OH was condensed with Boc–Leu–OH by the MA procedure to give Boc–Leu–Lys(Z)–OH (II), which, after conversion to the de-Boc peptide, was further condensed with Boc–Asp(OBzl)–OH by the MA procedure to give Boc–Asp(OBzl)–Leu–Lys(Z)–OH (III). The tripeptide III was treated with TFA and the product was also condensed with Boc–Lys(Z)–OH by the MA procedure to give Boc–Lys(Z)–Asp(OBzl)–Leu–Lys(Z)–OH (IV). The undecapeptide I was treated with TFA and the product was condensed with IV by the HONB–DCC procedure to minimize undesirable racemization to provide Boc–Lys(Z)–Asp(OBzl)–Leu–Lys(Z)–Glu(OBzl)–Lys(Z)–Lys(Z)–Glu(OBzl)–Val–Val–Glu(OBzl)–Ala–Glu(OBzl)–Asn–ONb (V). N-Methyl-2-pyrrolidone had to be used as a solvent, because of the poor solubility of the amino component in DMF. After removal of the Boc group of V with TFA, the resulting pentadecapeptide ester was hydrogenated over 10% Pd–C in aqueous AcOH for 18 hr. The hydrogenated product was purified by partition column chromatography on Sephadex G-25 according to Yamashiro. A solvent system consisting of BuOH, AcOH, and H₂O (4: 1: 5) was used to elute the desired compound. The absorbancy (230 nm) due to the peptide bond was used as a guide in this chromatographic purification. Homogeneity of the synthetic pentadecapeptide thus obtained was confirmed by paper chromatography and amino acid analysis. The 5.5 N HCl hydrolysate contained the constituent amino acids in the ratios predicted by theory. In addition, complete digestion of the synthetic pentadecapeptide was achieved by aminopeptidase M (AP-M). Serum from a uremic patient with chronic renal failure was found to inhibit markedly lymphocyte transformation by PHA (Table I). After incubation with amounts of the pentadecapeptide ranging from 100 to 200 μg/ml of cell culture, the amount of ³H-thymidine incorporation into DNA was increased (Table I). The decapetide has no effect on the lymphocyte transformation-inhibiting activity of uremic serum at a dose of 200 μg/ml. These results suggest that the key residues involved

<table>
<thead>
<tr>
<th>Peptide</th>
<th>Dose (μg/ml)</th>
<th>³H-Thymidine incorporation (cpm)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>a,b,c</em></td>
<td></td>
<td>310 ± 30</td>
</tr>
<tr>
<td><em>b,c</em></td>
<td></td>
<td>34981 ± 3461</td>
</tr>
<tr>
<td><em>c,d</em></td>
<td></td>
<td>12658 ± 3645</td>
</tr>
<tr>
<td>H–Lys–Lys–Glu–Val–Val–Glu–Glu–Ala–Glu–Asn–OH&lt;sup&gt;c,d&lt;/sup&gt;</td>
<td>100</td>
<td>12489 ± 4136</td>
</tr>
</tbody>
</table>

a) PHA (−).  

b) Lymphocytes were incubated with normal serum (0.02 ml) for three days at 37°C.  
c) PHA (+).  
d) Lymphocytes were incubated with uremic serum (0.02 ml) for three days at 37°C.  

in the active site of thymosin $\varepsilon_1$ for agonistic activity towards lymphocyte transformation inhibition by uremic serum are present within our synthetic pentadecapeptide.

Experimental

Melting points are uncorrected. Rotations were determined in a Atago Polax (cell length: 10 cm). The amino acid composition of the acid and enzymatic hydrolysates were determined with a JEOI JLC-8AH amino acid analyzer. Solvents were removed by evaporation in vacuo at a bath temperature of 40 to 50$^\circ$C in a rotary evaporator. Boc groups of the protected peptides were deblocked with TFA. The resulting amino components were chromatographed on filter paper, Toyo Roshi No. 51, at room temperature. $R^2$ values refer to the Partridge system\textsuperscript{14} and $R^4$ values refer to BuOH-pyridine-AcOH-H$_2$O (30: 20: 6: 24).\textsuperscript{15} Uremic blood and T-lymphocytes were obtained from a uremic patient suffering from terminal chronic renal failure. The blood was centrifuged and the separated serum was stored at $-20^\circ$C until use. Control serum was obtained from a healthy person. AP-M was purchased from the Protein Research Foundation (Osaka).

Boc-Glu(Obzl)-Lys(Z)-Lys(Z)-Glu(Obzl)-Val-Val-Glu(Obzl)-Glu(Obzl)-Ala-Glu(Obzl)-Asn-0NB (I) -- Boc-Lys(Z)-Lys(Z)-Glu(Obzl)-Val-Val-Glu(Obzl)-Glu(Obzl)-Ala-Glu(Obzl)-Asn-0NB $^b$ (204 mg) was treated with TFA (1.0 ml) in the presence of anisole (0.1 ml) at room temperature for 30 min, and the TFA salt precipitated by addition of dry ether was collected by filtration and dried over KOH pellets in vacuo. The powder was dissolved in ice-chilled DMF (3.0 ml) together with triethylamine (0.02 ml), HOBT (15 mg) and Boc-Glu(Obzl)-OH (317 mg). After addition of WSCI (18 mg), the mixture was stirred at 0$^\circ$C for 18 hr and poured into 1 N citric acid with vigorous stirring. The resulting precipitate was washed batchwise with 1 N citric acid, H$_2$O, 1 N NaHCO$_3$ and H$_2$O and then recrystallized from hot MeOH; yield 52 mg (71%), mp 198-206$^\circ$, $[\alpha]^{D}_{D} -21.0^\circ$ ($c=1.0$, DMF), $R^2$ 0.83, $R^4$ 0.91, single ninhydrin-positive spot. Anal. Calcd for C$_{11}$H$_{14}$N$_4$O$_5$: C, 62.25; H, 6.48; N, 9.31. Found: C, 62.14; H, 6.66; N, 9.48.

Boc-Leu-Lys(Z)-OH (II) -- A mixed anhydride prepared from Boc-Leu-OH (1.3 g) with N-methylmorpholine (0.64 ml) and ethylchlorocarbonate (0.51 ml) at $-10^\circ$ in tetrahydrofuran (THF) (5 ml) and acetonitrile (5 ml) was added to a cold solution of H-Lys(Z)-OH (1.3 g) in DMF (5 ml). The solution was stirred in an ice-bath for 6 hr, then the solvent was removed and the residue was dissolved in EtOAc. The solution was washed successively with 1 N citric acid and H$_2$O, dried over MgSO$_4$ and concentrated. The residue was repurified from EtOAc and n-hexane; yield 1.3 g (52%); mp 51-53$^\circ$, $[\alpha]^{D}_{D} -50.0^\circ$ ($c=1.0$, DMF), $R^2$ 0.68, $R^4$ 0.85, single ninhydrin-positive spot. Anal. Calcd for C$_{14}$H$_{18}$N$_4$O$_3$: C, 60.58; H, 8.34; N, 8.49. Found: C, 60.21; H, 8.65; N, 8.10.

Boc-Asp(Obzl)-Leu-Lys(Z)-OH (III) -- The above protected dipeptide (993 mg) was treated with TFA (4 ml) in the presence of anisole (0.5 ml) as usual and n-hexane was added. The resulting oil was dried over KOH pellets in vacuo, and dissolved in DMF (5 ml) containing N-methylmorpholine (0.2 ml). To this ice-chilled solution, the mixed anhydride (prepared from 647 mg of Boc-Asp(Obzl)-OH with 0.21 ml of ethylchlorocarbonate and 0.2 ml of N-methylmorpholine at $-10^\circ$ in THF (5 ml) and acetonitrile (5 ml) was added. The solution was stirred at 4$^\circ$C for 6 hr, then concentrated, and the residue was diluted with EtOAc. The solution was washed as described above and then precipitated from EtOAc and n-hexane; yield 1.0 g (71%), mp 48-56$^\circ$, $[\alpha]^{D}_{D} -17.0^\circ$ ($c=1.0$, DMF), $R^2$ 0.74, $R^4$ 0.84, single ninhydrin-positive spot. Anal. Calcd for C$_{16}$H$_{18}$N$_4$O$_5$: C, 61.88; H, 7.21; N, 8.02. Found: C, 62.21; H, 7.52; N, 7.93.

Boc-Lys(Z)-Asp(Obzl)-Leu-Lys(Z)-OH (IV) -- Compound III (349 mg) was treated with TFA (1 ml)-anisole (0.1 ml) in the usual manner and the deprotected peptide was dissolved in DMF (1 ml) containing N-methylmorpholine (0.05 ml). To this ice-chilled solution, the mixed anhydride (prepared from 281 mg of Boc-Lys(Z)-OH with 0.1 ml of ethylchlorocarbonate and 0.05 ml of N-methylmorpholine at $-10^\circ$ in THF (2 ml) and acetonitrile (2 ml) was added. The solution was stirred at 4$^\circ$C for 6 hr, then concentrated, and the residue was diluted with EtOAc. The solution was washed as described above and then precipitated from EtOAc and n-hexane; yield 291 mg (61%); mp 47-51$^\circ$, $[\alpha]^{D}_{D} -24.5^\circ$ ($c=1.0$, DMF), $R^2$ 0.84, $R^4$ 0.89, single ninhydrin-positive spot. Anal. Calcd for C$_{18}$H$_{20}$N$_4$O$_5$: C, 61.33; H, 7.21; N, 8.58. Found: C, 61.54; H, 7.52; N, 9.01.

Boc-Lys(Z)-Asp(Obzl)-Leu-Lys(Z)-Glu(Obzl)-Lys(Z)-Glu(Obzl)-Val-Val-Glu(Obzl)-Glu(Obzl)-Ala-Glu(Obzl)-Asn-0NB (V) -- I (80 mg) was treated with TFA (1 ml)-anisole (0.1 ml) as described above. IV (45 mg), HONB (8 mg)\textsuperscript{11} and WSCI (7 ml) were added to an ice-chilled solution of the resulting undecapeptide ester trifluoroacetate in N-methyl-2-pyrrolidone (2 ml) followed by addition of N-methylmorpholine\textsuperscript{16} to keep the solution slightly alkaline. After 30 hr at 0$^\circ$, the reaction mixture was poured into 1 N NaHCO$_3$ with stirring. The precipitate thus formed was washed successively with 1 N NaHCO$_3$, H$_2$O, 1 N citric acid and H$_2$O. The precipitate was recrystallized from hot MeOH; yield 76 mg (61%), mp 181-195$^\circ$, $[\alpha]^{D}_{D} -37.3^\circ$.

Modification of Cytosine Moieties of Nucleic Acids with Hydrogen Sulfide (Nucleosides and Nucleotides. XXXIV)\(^1\)

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The chemical introduction of 4-thiouridine (S\(^4\)U) residues into nucleic acids was carried out by the reaction of nucleic acids with liquid hydrogen sulfide in aqueous pyridine, which caused the conversion of cytosine moieties to 4-thiouracil moieties.

**Keywords**—chemical modification; sulphydrolysis; hydrogen sulfide; 4-thiouridine; nucleic acids; yeast RNA; yeast tRNA; calf thymus DNA; ultraviolet absorption spectrum

It is important to investigate the chemical and physical properties of oligo- and polynucleotides containing 4-thiouridine (S\(^4\)U) in order to obtain information on the function of the S\(^4\)U residue in tRNA. Therefore, it seems desirable to be able to synthesize oligonucleotides containing S\(^4\)U by a simple procedure.


\(^2\) Location: Kita-ku, Sapporo, 060, Japan.