STRUCTURAL REQUIREMENT FOR THE BIOLOGICAL ACTIVITY OF SERUM THYMIC FACTOR

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1. Introduction

Several polypeptides have been claimed for thymic factors (hormones) which mediate the differentiation of T lymphocyte precursors to more matured T cells [1]. Bach et al. [2] have obtained a circulating thymic factor from pig serum and shown its thymus origin. This factor has been reported to exhibit various immunological activities including θ-conversion in vitro and in vivo [3], induction of suppressor T cells in NZB mice [4], normalization of the abnormally high level of autologous erythrocyte-binding cells in ATx mice [5]. The chemical structure of this factor, designated Facteur Thymique Sérique (FTS), was elucidated as a nonapeptide, <Glu–Ala–Lys–Ser–Gln–Gly–Gly–Ser–Asn-OH [6].

There exist few reports on the influence of the chemical structure of this factor on the biological activity [7,8]. Here, we describe the θ-conversion activity of synthetic FTS and its short chain analogs. The pentapeptide moiety (Lys–Ser–Gln–Gly–Gly) is shown to be a minimum essential part for the expression of full agonistic activity and the C-terminal part may have an enhancing role in the binding of this pentapeptide portion.

2. Materials and methods

Male C3H/He mice (6–9 weeks old) were used. Adult thymectomy was performed at 5–6 weeks. Azathiopurine (AZ) was purchased from Sigma Chemical Co. and used as the sodium salt. Anti-θ serum (AθS, AKR anti-C3H (Thy-1 · 2) γ-globulin) was obtained from Litton Bionetics.

The peptides were synthesized by a conventional method (to be reported elsewhere) and are listed in table 1. The homogeneity of the peptide was ascertained by paper electrophoresis, thin-layer chromatography and high-performance liquid chromatography.

2.1. *Rosette inhibition assay*

The rosette technique and rosette inhibition protocols in [9] were followed. Mice were used 10–30 days after adult thymectomy for this assay.

2.2. *Cytotoxic assay*

Theta-negative pre-T cells in normal mouse spleen were enriched in A plus B layers by discontinuous bovine serum albumin gradient centrifugation [10] and incubated with FTS peptide at 37°C for 2 h. The percentage of θ-bearing cells was evaluated by a cytotoxicity test using AθS plus complement [11]. Dead cells were enumerated by a trypan blue dye exclusion test.

3. Results and discussion

The peptides were examined in vitro on the ability to convert the relatively AZ-resistant cells from ATx mouse spleen to θ-positive rosette-forming cells that are more susceptible to AZ [12]. Since all the active analogs are full agonists, the activity is expressed by the affinity, i.e., the half inhibitory concentration (h.i.c.) defined as the concentration at which 50% of rosette-forming cells were inhibited by AZ (10 μg/ml) and the results are shown in table 1. (Des-<Glu^1)-FTS is strongly active like FTS, with h.i.c. $\sim 10^{-12}$ M. This

Abbreviations: FTS, serum thymic factor (Facteur Thymique Sérique); θ, theta (Thy-1) antigen; ATx, adult thymectomized; AZ, azathiopurine; <Glu, L-pyroglutamic acid; Aac, acetoacetyl

Table 1
Biological activity of FTS and the analogs

	Abbreviation	Activity	
		Rosette inhibition[a]	Cytotoxic[b]
1 2 3 4 5 6 7 8 9 <Glu-Ala-Lys-Ser-Gln-Gly-Gly-Ser-Asn-OH	FTS	1.5×10^{-12}	1.5×10^{-8}[c]
<Glu-Ala-Lys-Ser-Gln-Gly-Gly-Ser-OH	(des-Asn^9)-FTS	1.3×10^{-11}	2.1×10^{-7}
<Glu-Ala-Lys-Ser-Gln-Gly-Gly-OH	(des-Ser^8-Asn^9)-FTS	1.9×10^{-10}	1.3×10^{-7}
<Glu-Ala-Lys-Ser-Gln-Gly-OH	(des-Gly^7-Asn^9)-FTS	3.0×10^{-9}	$> 1 \times 10^{-4}$
<Glu-Ala-Lys-Ser-Gln-OH	(des-Gly^6-Asn^9)-FTS	$> 1 \times 10^{-6}$	$> 1 \times 10^{-4}$
<Glu-Ala-Lys-Ser-OH	(des-Gln^5-Asn^9)-FTS	$> 1 \times 10^{-6}$	$> 1 \times 10^{-4}$
H-Ala-Lys-Ser-Gln-Gly-Gly-Ser-Asn-OH	(des-<Glu^1)-FTS	1.5×10^{-12}	1.5×10^{-8}
H-Lys-Ser-Gln-Gly-Gly-Ser-Asn-OH	(des-<Glu^1-Ala^2)-FTS	1.7×10^{-10}	2.1×10^{-7}
H-Ser-Gln-Gly-Gly-Ser-Asn-OH	(des-<Glu^1-Lys^3)-FTS	$> 1 \times 10^{-6}$	$> 1 \times 10^{-4}$
Aac <Glu-Ala-Lys-Ser-Gln-Gly-Gly-Ser-Asn-OH	(N^{ϵ}-Aac-Lys^3)-FTS	$> 1 \times 10^{-6}$	$> 1 \times 10^{-4}$

[a] Activity is shown in terms of the half inhibitory concentration (h.i.c., see text for definition) of peptide
[b] Activity is shown in terms of the peptide concentration giving a half-maximum response
[c] Maximum θ-conversion is obtained at 4.3×10^{-8} M

indicates that the N-terminal pyroglutamyl residue is not necessary for the activity. The Lys^3 residue is indispensable for the activity because the elimination of this residue gives a completely inactive peptide ((des-<Glu^1–Lys^3)-FTS). A similar observation was made with the same analog [7]. The acylation of ϵ-amino group of Lys^3 has also abolished the activity. Progressive removal of C-terminal 3 residues (Gly^7, Ser^8 and Asn^9) decreases the affinity by factors of about 10 with each elimination. This is in contrast to [8], where complete disappearance of activity with the removal of Asn^9 residue was shown. Much shorter peptides such as (des-Gly^6–Asn^9)-FTS are inactive at the doses tested ($\leqslant 10^{-6}$ M).

The θ-conversion was also determined directly by a cytotoxic assay [11]. Since all active analogs gave the similar range of maximum response (14 ± 2%), the activity is evaluated by the concentration giving a half-maximum θ-conversion (table 1). The results are almost comparable with those in the rosette inhibition assay, though much higher peptide concentration is required to detect the activity, reflecting the low sensitivity of cytotoxic assay with respect to θ-conversion (Des-<Glu^1–Ala^2)-, (des-Asn^9)- and (des-Ser^8–Asn^9)-FTS show ~10% of FTS activity, but in this assay system (des-Gly^7–Asn^9)-FTS which is a full agonist in the rosette inhibition assay (table 1) does not induce a significant θ-conversion. The remainders of the analogs are inactive even at 10^{-4} M.

Inactive analogs lacking C-terminal few residues are shown to antagonize the action of FTS, whereas another inactive peptide devoid of Lys^3 residue ((des-<Glu^1–Lys^3)-FTS) has no inhibitory property toward FTS even at 2×10^{-8} M (fig.1). The Lys^3 residue may be involved both in the exertion of the biological activity and in the binding of FTS to the receptor site on a target cell.

As can be seen from table 1, the pentapeptide portion (Lys–Ser–Gln–Gly–Gly) is apparently a minimum essential part of FTS molecule which bears the full θ-inductive information. The C-terminal portion (Ser^8–Asn^9) and also the Ala^2 residue may act to enhance the binding of rest part of the molecule. Similar but more sophisticated example has been documented in the case of ACTH [13]. This view was further tested by two inactive analogs, one devoid

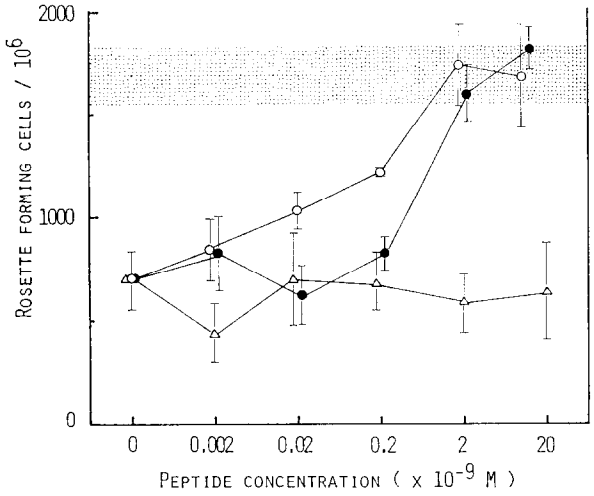

Fig.1. Inhibition of FTS activity by short chain analogs. FTS (0.005 × 10^{-9} M) was incubated together with either (des-Gln^5–Asn^9)-FTS (–○–), (des-Gly^6–Asn^9)-FTS (–●–) or (des-<Glu^1–Lys^3)-FTS (–△–) in the presence of AZ (10 μg/ml) and ATx mouse spleen cells. Dotted area corresponds to 2 SD of the control value without FTS.

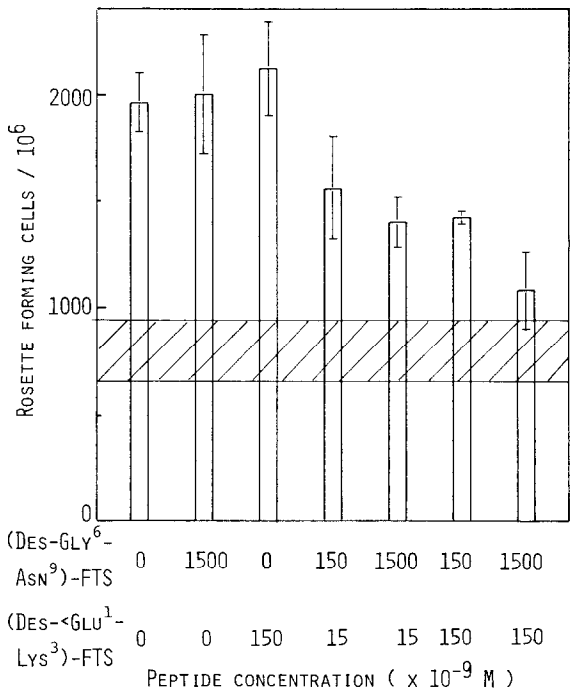

Fig.2. Restoration of FTS like activity by two inactive short chain analogs. The mixture of indicated concentration of (des-Gly^6–Asn^9)-FTS and (des-<Glu^1–Lys^3)-FTS was directly subjected to the rosette inhibition assay. Hatched area indicates 2 SD of the value with FTS (0.005 × 10^{-9} M).

of C-terminal part and the other devoid of N-terminal few residues. When the mixture of (des-Gly^6–Asn^9)-FTS and (des-<Glu^1–Lys^3)-FTS were subjected to the rosette inhibition assay, a significant biological activity was restored as shown in fig.2. Simultaneous attack by two inactive peptides may compensate each other the Lys^3 residue and C-terminal portion and they mimic the active conformation on a receptor site so that they could provide a θ-inductive signal. The observed low affinity (fig.2) can be explained by the rare opportunity of correct hit by the two molecules. The circular dichroism measurements of FTS and the analogs indicate their structural flexibility in water (unpublished). FTS may have a particular conformation on binding to the receptor site on a target cell. The results of this work agree most with a sychnological organization of θ-inductive information for FTS rather than a case of rhenylogical organization [14].

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