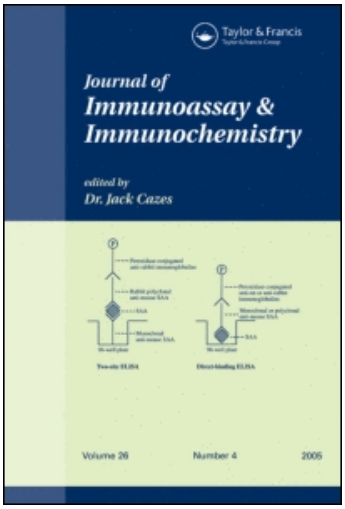

Journal of Immunoassay and Immunochemistry

Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597271

Radioimmunoassay of Serum Thymic Factor (FTS)

S. Ikeyama[a]; K. Kato[a]; M. Takaoki[a]; M. Takeuchi[a]; A. Kakinuma[a]
[a] Biotechnology Laboratories, Central Research Division, Takeda Chemical Industries, Ltd., Osaka, Japan

To cite this Article Ikeyama, S. , Kato, K. , Takaoki, M. , Takeuchi, M. and Kakinuma, A.(1984) 'Radioimmunoassay of Serum Thymic Factor (FTS)', Journal of Immunoassay and Immunochemistry, 5: 1, 87 — 97
To link to this Article: DOI: 10.1080/01971528408063000
URL: http://dx.doi.org/10.1080/01971528408063000

JOURNAL OF IMMUNOASSAY, 5(1&2), 87-97 (1984)

RADIOIMMUNOASSAY OF SERUM THYMIC FACTOR (FTS)

S. Ikeyama, K. Kato, M. Takaoki, M. Takeuchi and A. Kakinuma
Biotechnology Laboratories, Central Research Division, Takeda Chemical Industries, Ltd., Yodogawa-ku, Osaka 532, Japan

ABSTRACT

We established a radioimmunoassay (RIA) method which enables a quantitative estimation of serum thymic factor (FTS). This assay is based on the displacement of ^{125}I-(Lys[Tyr]^{3})-FTS bound to the anti-FTS antibodies by FTS. Formaldehyde-fixed Staphylococcus aureus Cowan I cells were used to precipitate the antigen-antibody complex. The anti-FTS antiserum was raised in rabbit by repeated injections of FTS-conjugated bovine serum albumin. The antiserum seemed to recognize the carboxy-terminal seven amino acid residues, Lys-Ser-Gln-Gly-Gly-Ser-Asn-OH, of the FTS molecule. As little as 0.5 to 1 pg FTS can be detected by this method.

To measure the FTS content in the serum, a serum sample was pretreated with 10% trichloroacetic acid and then with ethanol for removal of serum proteins. The FTS content in pig serum was around 22 pg/ml, and that in BALB/c mouse serum was 1.3 pg/ml. The FTS contents in the sera of DBA/2, C57BL/6 and (C57BL/6 x DBA/2)F_1 mice were also less than 5 pg/ml. Since more than 70% of FTS exogenously added to pig or mouse serum was recovered, the mouse serum probably contains only a small amount of FTS.

KEY WORDS: Serum thymic factor (FTS), Pig and mouse sera, Radioimmunoassay

INTRODUCTION

Bach et al. (1) found a thymic hormone which they called serum thymic factor (FTS). The structure of FTS isolated from pig serum was

 0197-1522/84/0501-0087$3.50/0

determined to be <Glu-Ala-Lys-Ser-Gln-Gly-Gly-Ser-Asn-OH (2,3). The contents of FTS in pig and mouse sera were determined by radioimmunoassay (RIA) and biological activities of FTS and its roles in maturation of T cells were investigated (4). They found that FTS was present in both thymus extracts and pig and mouse sera and that there was little FTS in the sera of thymectomized animals.

We have established a more sensitive RIA method using Staphylococcus aureus Cowan I cells and determined the FTS contents in pig and mouse sera. While an abundant amount of FTS was detected in pig serum, only a small amount was detected in sera of BALB/c, DBA/2, C57BL/6 and (C57BL/6 x DBA/2)F_1 mice.

MATERIALS AND METHODS

Chemicals

Chemically synthesized FTS (5), and its analogues listed in TABLE 1 including (Lys[Tyr]^3)-FTS and a fragment peptide of ubiquitin (amino acid residues from 59 through 74) were provided by Dr. M. Fujino of our Central Research Division. Chemically synthesized thymosin α_1 (6) was a kind gift from Hoffmann-La Roche Inc.,U.S.A.. Radiolabelling of (Lys[Tyr]^3)-FTS was performed by the chloramin-T method (7) and the labelled product was purified by gel filtration on a Sephadex G-10 column.

Sera

The mouse sera were obtained from 5 to 10 weeks old male and female BALB/c, DBA/2, C57BL/6, (C57BL/6 x DBA/2)F_1(BDF$_1$) and

TABLE 1

Binding-Inhibition Activity of FTS and Analogues.

Compound	Structure	Activity[a]
FTS	<Glu-Ala-Lys-Ser-Gln-Gly-Gly-Ser-Asn-OH	25
(Lys[Tyr]^3-FTS	<Glu-Ala-Lys(Tyr)-Ser-Gln-Gly-Gly-Ser-Asn-OH	25
Des(<Glu^1)-FTS	H-Ala-Lys-Ser-Gln-Gly-Gly-Ser-Asn-OH	80
Des(<Glu^1,Ala^2)-FTS	H-Lys-Ser-Gln-Gly-Gly-Ser-Asn-OH	72
Des(<Glu^1,Ala^2,Lys^3)-FTS	H-Ser-Gln-Gly-Gly-Ser-Asn-OH	450
Des(Asn^9)-FTS	<Glu-Ala-Lys-Ser-Gln-Gly-Gly-Ser-OH	no inhibition[b]
Des(Asn^9)-FTS isobutyrate	<Glu-Ala-Lys-Ser-Gln-Gly-Gly-Ser-NHCH$_2$CH(CH$_3$)$_2$	no inhibition[b]
(D-Ala^2)-FTS	<Glu-D-Ala-Lys-Ser-Gln-Gly-Gly-Ser-Asn-OH	34
(D-Ala^4)-FTS	<Glu-Ala-Lys-D-Ala-Gln-Gly-Gly-Ser-Asn-OH	>600[c]
(Thr^5)-FTS	<Glu-Ala-Lys-Ser-Thr-Gly-Gly-Ser-Asn-OH	>600[c]
(D-Ala^6)-FTS	<Glu-Ala-Lys-Ser-Gln-D-Ala-Gly-Ser-Asn-OH	>600[c]
(D-Leu^7)-FTS	<Glu-Ala-Lys-Ser-Gln-Gly-D-Leu-Ser-Asn-OH	>600[c]
(Ala^8)-FTS	<Glu-Ala-Lys-Ser-Gln-Gly-Gly-Ala-Asn-OH	600
(Ala^9)-FTS	<Glu-Ala-Lys-Ser-Gln-Gly-Gly-Ser-Ala-OH	no inhibition[b]

a Amount of compound (p mole) for causing 50% binding-inhibition.

b B/B_0 at a concentration of 600 p mole/tube was above 0.9.

c B/B_0 at a concentration of 600 p mole/tube was between 0.5 and 0.9.

BALB/c-nu/nu mice bred and maintained at our Central Research Division. The pig serum was obtained from a one week old animal.

The anti-FTS antiserum was raised in rabbit by repeated subcutaneous injections of FTS-conjugated bovine serum albumin as described previously (8).

Pretreatment of serum

The pooled serum (3 ml) was mixed with 3 volumes of ice-cold distilled water and 1 volume of 50% trichloroacetic acid (TCA). After the mixture was stood for 1 hr at 0°C, the precipitate was removed by centrifugation. The supernatant was vigorously mixed with an equal volume of ice-cold ethyl ether and the ether layer was removed. After this ether extraction was repeated 5 times, 0.1 ml of phosphate buffered saline (PBS) was added to the water layer in order to neutralize it. The treated serum was lyophilized and the residue on lyophilization was dissolved in distilled water of the original serum volume. The solution was mixed with 6 volumes of ethanol and kept for 2 hr at 0°C. After centrifugation, the supernatant was concentrated under N_2 stream and lyophilized. The residue was dissolved in 0.2 ml of NET buffer (150 mM NaCl, 5 mM EDTA, 50 mM Tris, 0.02% NaN_3 and 2 mM phenylmethyl sulfonylfluoride (pH 7.4)) containing 0.05% NP-40 and 0.1% ovalbumin (OVA), and the mixture was applied on a 5 ml Sephadex G-10 column which had been equilibrated with the same buffer. The first 1.5 ml eluate was discarded, and the next 1.5 ml was collected and used for the measurement of FTS by RIA.

RIA

1) Measurement of the binding-inhibition activity of FTS and its analogues.

Binding-inhibition curves for FTS and its analogues were obtained as folllows. The anti-FTS antiserum (4 μl) diluted with NET buffer containing 0.05% NP-40 and 0.1% OVA was mixed with ^{125}I-(Lys[Tyr]^{3})-FTS (29,000 cpm/9.5 ng) and FTS or its analogues. The whole mixture (500 μl) was stood overnight at 4°C. Heat-inactivated and formaldehyde-fixed whole cells of Staphylococcus aureus Cowan I (9) (5 μl in packed cell volume) were added to the mixture, and the preparation was kept for 60 min at 0°C. The precipitate obtained after centrifugation for 15 min at 4°C was washed twice with 500 μl of NET buffer containing 0.05% NP-40 and 0.1% OVA. The radioactivity in the precipitate was determined using an automated gammaspectrometer. Determination was carried out in triplicate for each sample.

2) Measurement of the FTS content in the serum.

For the measurement of the FTS content in the serum, a serum sample was pretreated as described above but the RIA conditions were slightly modified: the amounts of anti-FTS antiserum and ^{125}I-(Lys[Tyr]^{3})-FTS were 0.01 μl (final concentration, 1:50,000) and 1 pg (5,100 cpm), respectively, the first incubation at 4°C was extended to 4 days , and the antigen-antibody complex was precipitated with 1.25 μl of Staphylococcus aureus packed cells.

RESULTS AND DISCUSSION

Binding-inhibition activity of FTS analogues

We investigated in detail the binding-inhibition activities of FTS analogues, the synthetic fragment peptide of ubiquitin (amino acid

residues from 59 through 74) and thymosin α1 by the RIA method based on the displacement of ^{125}I-(Lys[Tyr]^{3})-FTS bound to the anti-FTS antibodies. FIGURE 1 shows typical binding-inhibition curves. FTS and (Lys[Tyr]^{3})-FTS inhibited the binding of ^{125}I-(Lys[Tyr]^{3})-FTS to the antibodies, but des(Asn^{9})-FTS, the synthetic fragment peptide of ubiquitin, and thymosin α1 did not inhibit this binding even at concentrations as high as 600 p mole/tube. The concetrations of these substances and other FTS analogues causing 50% binding-inhibition are summarized in TABLE 1. Des(<Glu^{1},Ala^{2})-FTS produced a moderate decrease of the binding of ^{125}I-(Lys[Tyr]^{3})-FTS. Des(<Glu^{1},Ala^{2},Lys^{3})-FTS produced a marked decrease of the binding. The binding-inhibition activity of des(<Glu^{1},Ala^{2},Lys^{3})-FTS was about 1/20 of that of FTS. Replacement of one amino acid at positions from 4 through 9 of FTS with other amino acid always caused a marked decrease of the activity. These results show that the anti-FTS antiserum recognizes the amino acid sequence from 3 to 9 of the FTS molecule, and that the specificity of this RIA method is very high. Recently, an anti-FTS antiserum which had a similar antigenic specificity to ours was reported by Fok et al. (10).

Measurement of the FTS content in the serum

We applied this RIA method for measurement of the FTS content in the serum. The reaction mixture (500 μl) containing 1 pg of ^{125}I-(Lys[Tyr]^{3})-FTS, anti-FTS antiserum (final concentration, 1:50,000) and FTS (0-16 pg) was incubated for 4 days at 4°c. A typical standard binding-inhibition curve is shown in FIGURE 2. The sensitivity of the method was 0.5-1 pg.

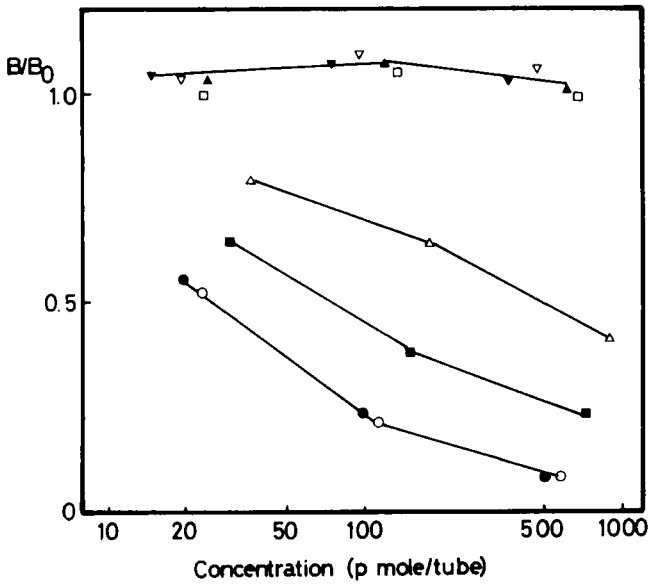

FIGURE 1. Binding-inhibition curves for FTS, its analogues, thymosin α1 and synthetic fragment peptide of ubiquitin (amino acid residues from 59 through 74). The reaction mixture (500 µl) containing anti-FTS antiserum (4 µl) and 9.5 ng of ^{125}I-(Lys[Tyr]^{3})-FTS was incubated in the presence of FTS (○), (Lys[Tyr]^{3})-FTS (●), des(<Glu^{1}, Ala^{2})-FTS (■), des(<Glu^{1},Ala^{2},Lys^{3})-FTS (△), des(Asn^{9})-FTS (□), (Ala^{9})-FTS (▲), thymosin α1(▽) or the synthetic fragment peptide of ubiquitin (▼), as described in text. B, radioactivity bound in the presence of unlabelled compounds; B_0, radioactivity bound in the absence of unlabelled compounds.

Before determinations, pig and mouse serum samples were treated with 10% TCA, ether, and ethanol successively to remove serum proteins. The FTS content in the pig serum was 22±8 pg/ml, while those in the sera from BALB/c, DBA/2, C57BL/6 and BDF_1 mice were always lower than 5 pg/ml (TABLE 2). The FTS contents in the sera of BALB/c mice of various ages were averagely 1.3±0.7 pg/ml and hardly changed with age and sex (TABLE 3). The FTS content in the serum of BALB/c-nu/nu mouse was lower than that of BALB/c mouse and below the sensitivity limit of the

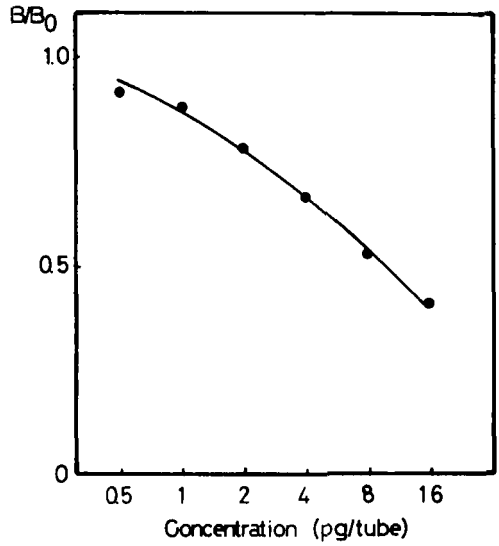

FIGURE 2. Standard binding-inhibition curve for FTS. The reaction mixture (500 µl) containing anti-FTS antiserum (final concentration, 1: 50,000) and 1 pg of ^{125}I-(Lys[Tyr]^{3})-FTS was incubated in the presence of 0-16 pg of FTS. RIA was carried out as described in text. B, radioactivity bound in the presence of FTS; B_0, radioactivity bound in the absence of FTS.

TABLE 2

Contents of FTS in Mice and Pig Sera and Recovery of Exogenously added FTS.

Serum from	FTS (pg/ml)			
	Exp. 1 Addition of FTS (40 pg/ml)		Exp. 2 Addition of FTS (25 pg/ml)	
	-a	+b	-a	+b
BALB/c	<3	50	4	22
DBA/2	<5	40	<4	23
C57BL/6	<5	32	N.T.[c]	N.T.[c]
BDF_1	<5	41	<4	17
Pig	27	81	16	59

a A serum sample(3 ml) was treated with 10% TCA, ether, and ethanol, successively. The treated sample was passed through a Sephadex G-10 column as described in text.

b FTS was exogenously added to the serum and the sample was treated in the same way.

c Not tested.

TABLE 3

Content of FTS in Sera of BALB/c Mice of different Age and Sex.

Age	Sex	FTS (pg/ml)	Mean+SD
6 weeks	F	0.8, 1.0	
3 months	F	1.2	1.3±0.7
6 months	F	2.6	
6 months	M	1.1	

A serum sample (10 ml) was treated as described in the footnote a for TABLE 2.

method (data not shown). Several independent experiments using various mice and pig sera gave the reproducible results.

Recovery of the exogenously added FTS

To ascertain the recovery of FTS by pretreatment of the serum, FTS was exogenously added to either pig or mouse serum (25 and 40 pg/ml), and the serum was immediately treated as described in MATERIALS AND METHODS. The exogenously added FTS was almost totally recovered in the soluble fraction obtained after pretreatment of the serum (TABLE 2). As the exogenously added FTS was fully recovered from the mouse serum even after incubation for 2 hr at 22°C (data not shown), the loss and decomposition of FTS in the serum can be ruled out in these processes of pretreating the serum. Based on these observations it is likely that the mouse serum contains only a small amount of FTS.

Bach et al. (4) reported that the FTS contents in the pig and mouse sera were 32 and 45 pg/ml, respectively. In this paper we obtained almost the same value for the pig serum, but we could detect only a small amount of FTS in the mouse serum. This may not be due to the existence of FTS-

binding substances in the mouse serum, because the exogenously added FTS was recovered almost completely. As for the discrepancy of the results for the FTS content in the mouse serum, the difference in specificity between their antiserum and ours might be a cause for it, although there remains a possibility that our antiserum used in this paper recognized the pig FTS more specifically than the mouse FTS because of a probable difference(s) in their structures.

ACKNOWLEDGEMENTS

We are grateful to Dr. M. Fujino for the supply of FTS and its analogues. We thank Dr. Y. Sugino for helpful suggestions and encouragement.

REFERENCES

1. Bach J. -F., Dardenne M., Pleau J. -M. and Bach M. -A. Isolation, biochemical characteristics, and biological activity of a circulating thymic hormone in the mouse and in the human. Annals N. Y. Acad. Sci. 1975; 249: 186-199.

2. Dardenne M., Pleau J. -M., Man N. K. and Bach J. -F. Structural study of circulating thymic factor: a peptide isolated from pig serum I. Isolation and purification. J. Biol. Chem. 1977; 252: 8040-8044.

3. Pleau J. -M., Dardenne M., Blouquit Y. and Bach J. -F. Structural study of circulating thymic factor: a peptide isolated from pig serum. II. amino acid sequence. J. Biol. Chem. 1977; 252: 8045-8047.

4. Bach J. -F., Bach M.-A., Bianot D. et al. Thymic serum factor (FTS). Bull. Inst. Pasteur 1978; 76: 325-398.

5. Fukuda T., Kitada C. and Fujino M. p-Tolylmethylsulphonyl: a new amino acid-protecting group in peptide synthesis. J. Chem. Soc. Chem. Commun. 1978: 220-221.

6. Low, T. L. K., Thurman, G. B., McAdoo, M., McClure, J., Rossio, J. L., Naylor, P. H. and Goldstein A. L. The chemistry and biology of

thymosin. I. Isolation, characterization, and biological activities of thymosin α1 and polypeptide β1 from calf thymus. J. Biol. Chem. 1979; 254: 981-986.

7. Brown J. P., Kitzman J. M. and Hellstrom K. E. A microassay for antibody binding to tumor cell surface antigens using ^{125}I-labelled protein A from Staphylococcus aureus. J. Immunol. Methods 1977; 15: 57-66.

8. Kato K., Ikeyama S., Takaoki M., Shino A., Takeuchi M. and Kakinuma A. Epithelial cell components immunoreact with anti-serum thymic factor (FTS) antibodies: possible association with intermediate-sized filaments. Cell 1981; 24: 885-895.

9. Kessler S. W. Cell membrane antigen isolation with the Staphylococcal protein A-antibody absorbent. J. Biol. Chem. 1976; 117: 1482-1490.

10. Fok K. M., Ohga K., Incefy G. S. and Erickson B. W. Antigenic specifity of two antibodies directed against the thymic hormon serum thymic factor (FTS). Mol. Immunol. 1982; 19: 1667-1673.