Reprint from: Chemistry of Peptides and Proteins, Vol. 1
Editors: W. Voelter, E. Wünsch, J. Ovchinnikov, V. Ivanov

ISOLATION, PHYSICO-CHEMICAL AND BIOLOGICAL PROPERTIES OF THE IMMUNITY POLYPEPTIDE BIOREGULATOR FROM THYMUS

O.A.Pisarev, V.G.Morosov, V.Kh.Khavinson, L.K.Shataeva, G.V. Samsonov

Institute of Macromolecular Compounds, Academy of Sciences of the USSR, Leningrad

Introduction

The main subject of modern immunology is to investigate the ways and means for the recovery of the disturbed immunological functions in the process of human vital activity. One of the hypotheses accounting for a T-lymphocyte formation is the assumption that in thymus a hormonal factor is produced, due to its action the marrow stem cells obtain T-lymphocyte properties and are further differentiated into T-cells participating in immunological reactions (1). Thymosin is a well-known isolated thymus factor (2). There are some reports on the thymic humoral factor isolation (3), thymopoietin (4) as well as the circulating blood thymus factor (5). This study had the aim of the isolation of a polypeptide fraction stimulating immunogenesis and the examination of its composition, physico-chemical and biological properties.

Results

The main stages of the compound production from thymus are represented in Fig.1. Before the stage of the ion-exchange chromatography more than 10 compounds of different origin were present. The multi-component extract was fractionated on Biocarb (6-8) into three fractions; electrophoresis on polyacrylamide gel showed that in fraction one there are nine com-

pounds, in fraction 2 3 compounds and in fraction 3 there is 1 compound present. Fraction 1 eluates from the ion-exchange column at pH 3.0, fraction 2 at pH 6.5, fraction 3 at pH 9.5 - 10.0.

thymus tissue
acetone
(-4°, 48h)
homogenization
extraction
(6 x 3 % CH_3COOH + $ZnCl_2$, 72 h)
centrifugation
acetone
(-4°, 1 h)
dissolution in 5 % CH_3COOH
ion-exchange chromatography (Biocarb-T)
thymalin

Fig.1. Scheme of the isolation of thymalin.

According to gel chromatography on Sephadex G-25 and G-10 fraction 3 was also homogeneous. Investigations by ultracentrifigation demonstrated a symmetrical peak for fraction 3 with the sedimentation coefficient 0.7 Sw.

The percentages of each fraction, molecular masses, isoelectric points, tyrosine contents (by Lowry) and also the results of biological testing are shown in Figs. 2 and 3.

Fraction 1 has no biological activity. Fraction 2 stimulated immunological reactions 15-20 times stronger than compared to the extract. The biological activity of the extract is associated mainly with fraction 3. Intramuscular injection (0.5 mg/kg) of this fraction to the mouse increased the number of lymphocytes in blood by 50-70 %, and 0.1-0.5 mg/kg stimulated the formation of antibody forming cells threefold. At higher dose (25 mg/kg) this fraction had no stimulating effect and in some cases suppresed immunological reactions. Fraction 3 was named "thymalin" and was later used for clinical and bio-

logical tests.

Preparation	Dose mg/kg	Number of lymphocytes	Number of circulating antibody	Number of antibody forming cells	Phagocytic reaction	Activity of peroxidase
Fraction 1	25	-	-	-	-	-
	2.5	-	-	-	-	-
Fraction 2	25	±	±	-	±	±
	2.5	-	-	-	-	-
Fraction 3	25	+	±	-	±	±
	0.5	+	+	+	+	+
	0.1	+	±	+	±	+

Fig. 2. Effect of thymus preparations on the indices of immunologic reactivity.

Component	Content in extract (%)	Mol.	Isoelectr. points	Tyrosine content by Lowry %
Fraction 1	80	700	2.5	4.5
Fraction 2	15	5000	4.5	1.0
Fraction 3	5	5000	9.5	1.0

Fig. 3. Properties of thymic fractions.

Study of the thymalin amino acid composition confirmed its polypeptide structure. Fig.4 shows that thymalin is a basic polypeptide, consisting of 38 amino acid residues. In the thymalin structure there are 11 basic and 4 acid amino acids. N-terminal analysis performed according to (9) demonstrated that N-terminal amino acids are lysine and alanine.

Indices	Thymalin properties
Mol.	50 00
Isoelectric point	9.5
Number of amino acid residues	38
Amino acid composition	Asp_2, Thr_2, Ser_2, Glu_2, Trp_1, Pro_3, Gly_3, Ala_4, Val_1, Met_1, Ile_1, Leu_1, Tyr_1, Cys_1, Phe_1, His_1, Lys_8, Arg_3

Fig. 4. Physico-chemical properties of thymalin.

Conformational properties of thymalin were examined by the ORD method. Thymalin undergoes a cooperative conformational transition in the 8.5 - 10.5 range with $[\alpha]_D^{410^\circ}$ changing from -230° to -165°. Estimates according to (10,11) reveal no ordered structure in the pH area of 4.0-8.5, whereas at pH 10.5 ca. 20 % α-helix is present.

The results obtained in the study of the thymalin effect on the immunity indices are given in Fig.5. The number of T- and B-lymphocytes was determined by E-POK and EAG-POK content in the lymphatic nodules and the spleen of the animals as described in (12). After thymectomy the number of caryocites, T-lymphocytes in the spleen,has decreased to a great extent. Cell composition in the lymphatic nodules has not changed. After thymalin injection the number of caryocites has completely recovered and the number of T- and B-lymphocytes has enhanced in the spleen. In the lymphatic nodules, the changes of caryocite content, T- and B-lymphocytes appeares less noticeable.

The effect of thymalin on T-cell population was confirmed by the investigation of its action in the culture of lymphocytes produced from thymectomyzed and pseudo-operated guinea pigs. The percentage of T-cells in the spleen months after the operation was 28.6 ± 2.9 for the pseudo-operated animals

Group of animals	Number of caryocites mln		Number of T-lymphocytes th/mg		Number of B-lymphocytes th/mg	
	Spleen	Lymphatic nodules	Spleen	Lymphatic nodules	Spleen	Lymphatic nodules
Pseudo-operated	58.5± 18.3	84.3± 7.2	158.4± 9.4	310.3± 19.9	342.5± 20.1	362.3± 27.3
Thymecto-mized	225.1± 16.2	69.1± 4.8	69.2± 12.0	278.1± 25.1	275.4± 23.2	372.2± 31.1
Thymecto-mized + thymalin	352.6± 16.8	85.2± 5.7	153.2± 7.6	295.5± 21.6	301.3± 25.8	385.2± 30.9

Fig. 5. Effect of the thymus factor on the indices of guinea pig immunity.

and 16.8 ± 20 for the thymectomized ones. When the thymus factor was applied the content of T-cells in the lymphocyte culture of thymectomized guinea pigs increased to the initial indices. The content of T-cells in the lymphocyte culture of the pseudooperated animals did not markedly change.

From the above data we assume that the decrease of the number of T-cells in the spleen after thymectomy occurs owing to T_1-population of lymphocytes in mature animals which are present mainly in thymus and spleen. Thymectomy of mature animals only slightly affects the cell composition of the lymphatic nodules containing mostly T_2 population of lymphocytes. Thus one can suggest that thymalin recovers the number of T-lymphocytes possessing a function of a helper in a certain stage of their development and thereby favours the definition of antigene and the induction of the cooperative immune response to thymus-depending antigenes. Due to the lack of thymus factor after thymectomy the number of T-lymphocytes decreases, this being the possible reason for supressed immunological reactivity of thymectomized animals.

Thymus-dependence of lymphocyte population as well as the helper activity of antigene-activated cells are evidently mediated by the presence of thymus factor on the surface of lymphocytes. Hence we may suggest that T-lymphocytes are the carriers of a thymus factor inducing a cooperative immune response to thymus-depending antigenes. The effect of the thymus factor on different physiological systems is likely to be associated with the function of T-cells which contain genetic information and they are the participants not only of immunologic but also of metabolic processes, such as the regeneration of tissues, the synthesis of protein and nucleic acids, carbohydrate and fat metabolism.

References

1. Burnet, F.M.: Lancet 1, 358-360 (1970).
2. Goldstein, A.L., Guna, A.: Proc.Natl.Acad.Sci. 69, 1800-1810 (1972).
3. Trainin, N., Small, M.: J.Exp.Med. 132, 885-897 (1970).
4. Basch, R., Goldstein, G.: Proc.Natl.Acad.Sci. 71, 1474-1478 (1974).
5. Bach, A.L., Dardenne, M., Pleau, M.A., Bach, M.A.: Ann.N. Y.Acad.Sci. 249, 186-210 (1975).
6. Morozov, V.G., Khavisnon, V.Kh., Pisarev, O.A.: Proc.Acad. Sci. USSR. 233, 491-494 (1977).
7. Pisarev, O.A., Shataeva, L.K., Samsonov, G.V.: Bull.Acad. Sci., USSR. 29(1), 37-42 (1980).
8. Pisarev, O.A., Shataeva, L.K., Samsonov, G.V.: J.Physical Chemistry, USSR. 54. 2391-2395 (1980).
9. Cray, W.R.: Methods in Enzymol.11, 469-475 (1967).
10. Moffit, W., Yang, J.T.: Proc.Natl.Acad.Sci.42, 596-600 (1956).
11. Shechter,E., Blout, E.: Proc.Natl.Acad.Sci. 51, 794-798 (1964).
12. Stadecker, M.J., Bishop, G., Wortiz, H.H.; J.Immunol.3, 1834-1837 (1973).