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The octapeptide corresponding to the region of the highest homology between α-interferon and thymosin-α_1 effectively competes with both cytokines for common high-affinity receptors on murine thymocytes

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The octapeptide corresponding to human interferon-α_2 (Hu IFN-α_2) sequence 131–138 has high affinity to murine thymocyte receptors ($K_d = 4.2 \times 10^{-12}$ M, about 700 receptors per cell). The peptide/receptor binding is inhibited by both Hu rIFN-α_2 ($K_i = 8.6 \times 10^{-10}$ M) and thymosin-α_1 (TM-α_1) ($K_i = 3 \times 10^{-7}$ M) as well as by the octapeptide homologous to the TM-α_1 sequence 16–23 ($K_i = 4.5 \times 10^{-7}$ M). The peptide from IFN-α_2 (131–138) activates murine thymocyte blast transformation at a concentration of 10^{-11} M in the presence of 2.5 μg/ml of concanavalin A.

Interferon-α; Thymosin-α_1; Common receptor

1 INTRODUCTION

Interferons are polyfunctional proteins possessing antiviral, antitumor and immunomodulatory activities [1]. In order to localize the active sites of these proteins we predicted the secondary and three-dimensional structures of IFNs-α, -β, -γ, -ω [2–7] from their amino acid sequences, and revealed the conservative amino acid residues by means of the analysis of 72 IFNs-α, -β, -ω [6,7]. Three conventional clusters of conservative positions were identified: the 'loop' one – residues 30–39; 'hydrophilic' – residues 59–92; and 'hydrophobic' – residues 123–144 [7]. It is reasonable to assume that the loop sites at the region of conservative clusters can directly participate in the formation of IFN active sites. Particular attention was drawn to the loop segment at the region of amino acid residues 130–140. This segment was shown [5–7] to have the highest homology with the thymus hormone, thymosin-α_1 (TM-α_1) [8]. It is reasonable to suppose that this sequence corresponds to the immunomodulatory site of IFNs similar, in some features, to TM-α_1.

We report here the synthesis of the peptides corresponding to the region of the highest homology between IFN-α_2 and TM-α_1 together with the study of their binding by murine thymocytes and investigation of their biological activity.

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2. MATERIALS AND METHODS

TM-α_1 [8] and the peptides corresponding to the segments of the highest homology between IFN-α_2 and TM-α_1 were obtained by the solid-phase synthesis. The peptides were purified by HPLC on a chromatograph (Gilson, France) using Zorbax C_8 columns (DuPont, USA). According to the optical data at 220 nm, the content of the bulk substance was found to be 99%. The peptide structure was confirmed by the amino acid analysis under standard conditions on a D500 amino acid analyzer (Durrum, USA). Optical rotation was measured with a 141M polarimeter (Perkin-Elmer, USA). Peptide iodination was carried out according to the procedure suggested by Ling et al. [9]. The labelled peptide was purified from radiolysed products and impurities by gel filtration using a 0.9 × 10 mm column of Sephadex G-10 (Pharmacia, Sweden) equilibrated with 0.05 M phosphate buffer, pH 7.5. Purity of the labelled peptide was tested by thin-layer chromatography on aluminium oxide glass plates with the solvent system, *n*-butanol/acetic acid/water (4:1:1), followed by autoradiography. The preparations obtained contained not more than 5% of degradation products. Radioactivity was measured with a Mini Gamma Counter (LKB, Sweden). For the analysis of specific binding, the cells (10^7 per ml) were incubated at 4°C for 40 min with radiolabelled IFN-α_2 octapeptide (concentration range from 10^{-12} to 10^{-7} M) in solution of 50 mM Tris-HCl buffer with bacitracin (0.1 mg/ml), trypsin inhibitor from soybean (0.1 mg/ml), EGTA (1 mM), PMSF (0.6 mg/l). Non-specific binding was determined in the presence of 10^{-5} M unlabelled IFN-α_2 peptide. The incubation was terminated by rapid filtration through GF/B glass fiber filters (Whatman, UK) under vacuum pressure. Filters were rinsed twice with 5 ml volumes of ice-cold buffer. Each experiment was repeated and all the samples were analyzed at least in duplicate.

For the analysis of inhibitory effects murine thymocytes (10^7–10^8 cells/ml) were incubated for 40 min at 4°C with unlabelled thymosin-α_1 (concentration range from 10^{-12} to 10^{-5} M) and 0.3 nM of radiolabelled IFN-α_2 octapeptide in solution of 50 mM Tris-HCl buffer with bacitracin (0.1 mg/ml), EGTA (1 mM), PMSF (0.6 mg/l) and trypsin inhibitor from soybean (0.1 mg/ml). The incubation was terminated by rapid filtration through GF/B glass fiber filters (What-

man, UK) under vacuum pressure. Filters were rinsed twice with 5-ml volumes of ice-cold buffer. Cells in duplicate tubes were assayed for each concentration, and each experiment was replicated 2–4 times.

The inhibition constant (K_i) was calculated from the equation [10]:

$$K_i = \frac{IC_{50}}{1 + [L]/K_d}$$

where [L] is the concentration of labelled ligand and K_d is the equilibrium constant. IC_{50} values were determined graphically (see Fig. 2).

For the analysis of blast-transforming activity of the peptides, thymocytes were cultivated for 72 h in 96-well plates. Each well contained 5×10^5 cells in 200 μl of RPMI-1640 medium (Gibco, UK) supplemented with 5% inactivated calf fetal serum, 5 mM Hepes, 20 mM glutamin and 100 units/ml of both penicillin and streptomycin. 18 h before termination of the cultivation, [^{3}H]thymidin in 1 μCi doses was added to each well. To assess the reaction efficiency, the stimulation index was determined as the ratio of the number of radioactive impulses in the experimental cells to that in the control ones (culture medium free of the sample under study). Con A (Serva, USA) at the concentration of 2.5 μg/ml was used as a mitogen. The sample of Hu rIFN-α_2 was kindly provided by Dr V. Bumyalis (Institute of Applied Enzymology, Vilnyus, Lithyania).

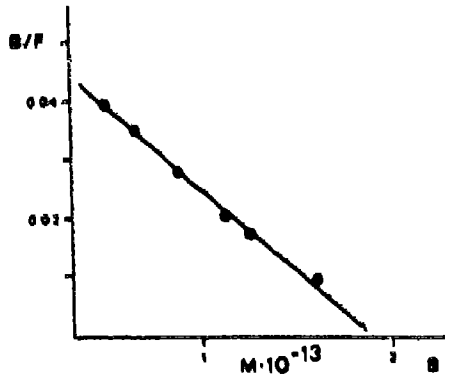

Fig. 1. Scatchard analysis of the specific binding of the ^{125}I-labelled octapeptide, LKEKKYSP (corresponding to the IFN-α_2 sequence 131–138) with murine thymocytes. B/F implies the ratio of molar concentration of the bound labelled peptide (B) to that of the free labelled octapeptide (F).

3. RESULTS

Table I, column 2 shows primary structures of the synthesized peptides, and column 1 indicates their origin. All the peptides share a common sequence, LKEKK. Parameters of binding with thymocyte receptors were determined for the peptides efficiently labelled with ^{125}I. The unlabelled peptides were studied in a test of inhibition of binding of the most active (among those studied) labelled IFN-α_2 peptide, LKEKKYSP.

The analysis of binding of the IFN-α_2 peptide with thymocytes by the method in [10] (Fig. 1) revealed one type of thymocyte receptor (Scatchard plot represents a direct line). The binding is characterized by high affinity ($K_d = 4.2 \times 10^{-12}$ M, see Table I) and by a relatively low density of binding sites per cell (~700). The unlabelled rIFN-α_2 and TM-α_1 inhibit the binding of the labelled IFN-α_2 octapeptide ($K_i = 8.6 \times 10^{-10}$ M and 3×10^{-7} M, respectively) (Fig. 2, Table I). Inhibitory activity of TM-α_1 is reproduced by the TM-α_1 16–23 octapeptide ($K_i = 4.5 \times 10^{-7}$ M, Table I). Extension of the active sequence with one residue from the C-terminus induces affinity decrease by a factor of 10^4 (Table I). Extension of the same sequence from the N-terminus results in a loss of binding capacity (Table I). The pentapeptide corresponding to the sequence LKEKK, shared by IFN-α_2 and TM-α_1, does not produce any inhibitory effect on the receptors (Table I). This indicates an important role of the sequence YSP for the interaction with receptors. Its replacement with the sequence EVV in TM-α_1 (16–23) significantly decreases the peptide binding capacity (Table I).

The IFN-α_2 octapeptide can be comparable with rIFN-α_2 in the reaction of blast transformation (Fig. 3). In the presence of low doses of Con A (2.5 μg/ml) the peptide at a concentration of 10^{-11} M induces

Table I

Parameters of binding of Hu rIFN-α_2, TM-α_1 and peptides, corresponding to the cytokine sequences, with thymocyte receptors

Peptide origin	Peptide sequence	K_d(M)	K_i(M)
Hu IFN-α_2	Hu rIFN-α_2	nd*	8.6×10^{-10}
IFN-α_2 (131–138)	LKEKKYSPC	6.2×10^{-8}	i
IFN-α_2 (131–138)	LKEKKYSP	4.2×10^{-12}	i
IFN-α_2 (131–135)	LKEKK	nd	ni***
IFN-α_2 (126–135)	RITLYLKEKK	nb**	ni
IFN-α_2 (123–135)	YFQRITLYLKEKK	nb	ni
IFN-α_2 Cys (Acm) (131–138)****	CLKEKKYSP	nb	ni
IFN-α_2 [(131–138) C-Cys]$_2$	LKEKKYSPC LKEKKYSPC	nb	ni
TM α_1	TM-α_1	nd	3×10^{-7}
TM-α_1 (16–23)	LKEKKEVV	nd	4.5×10^{-7}
TM-α_1 (11–20)	ITTKDLKEKK	nd	ni

*Non-determined
**Non-bound
***Non-inhibited
****Peptide dimerization is blocked by Cys modification with Acm- (acetamidomethyl) group

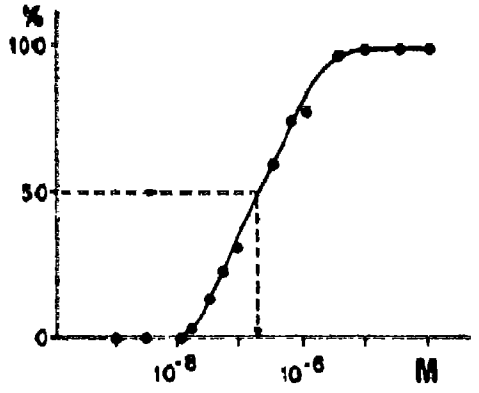

Fig. 2. Inhibitory effects of thymosin-α_1 on the binding of the ^{125}I-labelled IFN-α_2 (131–138) octapeptide. The arrow indicates the graphic mode of estimation of IC_{50} value.

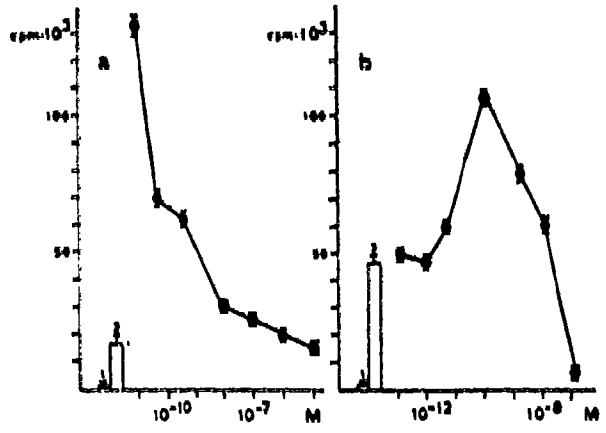

Fig. 3. Activation of murine thymocyte blast transformation by the octapeptide LKEKKYSP (a) and Hu rIFN-α_2 (b) in the presence of 0.25 μg/ml Con A. The quantity of radioactive impulses per minute (cpm, counter per minute) is plotted as the ordinate and molar concentrations of the octapeptide (a) and rIFN-α_2 (b) are plotted as the abscissa. Thymocytes were cultivated for 72 h in 96-well plates. Each well contained 5×10^5 cells in 200 μl of RPMI-1640 medium supplemented with 5% inactivated calf fetal serum, 5 mM Hepes, 20 mM glutamine and 100 units/ml of both penicillin and streptomycin. 18 h before termination of the cultivation, [^{3}H]thymidine in 1 μCi doses was added to each well. The control 1 was the culture medium free of the sample under study, and the control 2 was the culture medium + 2.5 μg/ml Con A used as a mitogen.

thymocyte blast transformation more efficiently than rIFN-α_2.

4. DISCUSSION

The experimental data obtained provide evidence that the sequence 131–138 of IFN-α_2 is responsible for binding to high-affinity receptors on murine thymocytes. The fact that Hu rIFN-α_2 effectively inhibits this reactivity indicates the lack of essential species-distinctions in IFN-α_2 binding by the given binding site. These receptors are common to IFN-α_2 and TM-α_1, since both cytokines exert an inhibitory effect on the binding of the radiolabelled octapeptide IFN-α_2 (131–138). In TM-α_1 the sequence 16–23 is responsible for this activity. Since binding of the IFN-α_2 octapeptide (131–138) with thymocyte receptors is characterized by a linear Scatchard plot, it can be concluded that the receptor identity is independent of a subpopulation of cells and extent of their differentiation. The binding of radiolabelled IFN-α to cells was studied in many tissue culture cells from both mice and humans [12]. A wide variety of cell types have a relatively low number (2×10^2 to 1×10^4) of high-affinity (K_d of 1×10^{-9} to 1×10^{-11} M) receptors for IFN-α. Many binding curves do not show true saturation resulting in curvilinear Scatchard plots. Some authors suggested that this reflects the presence of a fairly large number of low-affinity receptors in addition to the high-affinity ones [12]. Our results provide evidence that the sequence 130–140 of IFN-α corresponds to the high-affinity binding site of this protein. According to our unpublished data the IFN-α_2 peptide 131–138 does not reproduce antiviral activity of IFN but has some immunomodulatory properties in common. The IFN-α_2 fragment 131–138 seems to correspond only to a part of the immunomodulatory site of the given cytokine, since, at the molar level, it reproduces only the blast-transforming activity of IFN-α.

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