SYNTHETIC PEPTIDES FROM THE REGION 30-54 OF THE INTERLEUKIN-2 RECEPTOR β-CHAIN (IL-2Rβ) AFFECT IL-2 BINDING TO HUMAN T-CELLS

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The interleukin-2 receptor β-chain (IL-2Rβ) is responsible for the IL-2 internalization and signal transduction. To identify domains from the IL-2Rβ extracellular region which are involved in ligand–receptor interactions, eight peptides of 8 to 25 amino acids corresponding to IL-2Rβ sequence 30-54 were synthesized. Their biological effect was tested with [^{125}I]IL-2 equilibrium binding to PHA-blasts. Preincubation of [^{125}I]IL-2 with peptides IL-2Rβ (35-54) and (30-42) affects IL-2 binding to both high and intermediate affinity receptors by revealing a greater number of active sites with lower affinity.

IL-2 is a well-characterized lymphokine that plays a pivotal role for growth or differentiation of lymphocytes. To exert its biological effects IL-2 must interact specifically with high affinity cell surface receptors[1 – 3]. The high affinity receptor (IL-2R), with a dissociation constant (K_d) of ~100 pmol/l is composed of at least two distinct polypeptide chains; the α-chain of 55 kDa (IL-2Rα, p55) and the β-chain of 75 kDa (IL-2Rβ, p75). Unlike other multi-subunit receptors, each of the two chains can be individually expressed on the cell surface. Also, they interact with different regions of the IL-2 molecule and bind the ligand with low (K_d = 1 – 10 nmol/l) and intermediate (K_d = 0.2 – 1 nmol/l) affinities, respectively[4,5]. Recent studies provided evidence for a yet more complex structure for the IL-2R involving other molecules referred to as γ-subunit or p90-110 (ref.[6]) and p64 (ref.[7]). The latter molecule, whose primary structure was identified last year by Takeshita et al.[8], participates in the formation of both high and intermediate affinity IL-2R.

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It has been demonstrated that the α-chain itself can bind IL-2 but is not functional for signal transduction[9]. Furthermore, the internalization of IL-2 was only mediated by the high or the intermediate affinity receptors[10 – 12]. Thus, the β-chain is essential for the IL-2 signal transduction.

The primary amino acid sequence of the human IL-2Rβ was predicted from cloned cDNA's. The IL-2Rβ consists of an extracellular, a transmembrane and a cytoplasmic region, with 214, 25 and 286 amino acids, respectively[13]. There is evidence that the high affinity IL-2R is generated by non-covalent association between the two chains solely at their extracellular regions[14 – 15], and several models have been suggested concerning the formation of the IL-2/IL-2R complex[16,17]. However, little work has been performed to identify the IL-2Rβ residues that are involved in the IL-2 binding and its association with a-chain[18,19].

In an attempt to localize site(s) on the IL-2Rβ extracellular region which are essential for the interactions between IL-2 and its receptors, we synthesized and purified eight peptides derived from the hydrophilic region 30-54 of IL-2Rβ. Two synthetic peptides, containing residues 35-54 and 30-42, affect the binding of [^{125}I]IL-2 to human T-cells by revealing a higher number of receptors which bind IL-2 with lower affinity.

EXPERIMENTAL

Peptide Synthesis

Peptides were synthesized stepwise according to the solid phase method of Merrifield[20,21], on phenylacetamidomethyl (PAM) resins (Novabiochem). Synthetic protocols were based on the classical *t*-Boc/Bzl chemistry. The following side-chain protected amino acid derivatives were used: Boc-His(Bzl)*, Boc-Glu(OBzl), Boc-Arg(Mts), Boc-Cys(SBzl), Boc-Cys(MeBzl), Boc-Thr(Bzl), Boc-Gln(ONp), Boc-Asn(ONp), Boc-His(Tos) and Boc-Asp(OBzl). Boc protecting groups were removed at each stage by treatment with 45% trifluoroacetic acid (TFA) in dichloromethane (DCM) containing 1% anisole or ethanedithiol (EDT) for 25 min. Neutralization was effected with 10% triethylamine in DCM for 5 min. The removal of the resin-bound peptide was accomplished with HF containing 9% (v/v) *p*-cresol and 1% (v/v) EDT at < 0 °C for 1 h, and the peptides were extracted with 10 – 30% acetic acid. The sulfhydryl groups on the Cys(SBzl) residues still remained protected after the cleavage procedure. The crude peptides were desalted on a Sephadex G-15 or G-25 column using 2 M AcOH as the eluent. Fractions that gave single spots on TLC in the solvent systems butanol–AcOH–H_2O (4 : 1 : 5, v/v), or butanol–AcOH–H_2O–pyridine (15 : 3 : 8 : 10, v/v) were collected. Alternatively, peptides were further purified by HPLC on a reversed-phase C_{18} column (HPLC Techn., U.K., 10 × 250 mm) with a continuous gradient of 15 to 70% acetonitrile in 0.1% TFA at 214 nm, and found to have an average purity of 90%. The amino acid composition of individual peptides was confirmed by amino acid analysis on a Beckmann C-190 Analyzer.

* The nomenclature and symbols of amino acids obey the IUPAC/IUB Recommendations (Eur. J. Biochem. *138*, 9 (1984)).

IL-2Rβ(30-54): Asp: 2.90, Thr: 1.90, Ser: 2.10, Cys: 1.60, Glu: 3.10, Val: 2.00, His: 0.92, Ala: 1.15, Trp: 1.65, Pro: 2.08, Arg: 2.85, Leu: 2.10.

IL-2Rβ(40-50): Val: 1.10, His: 0.90, Ala: 1.05, Trp: 1.70, Pro: 2.10, Asp: 1.15, Arg: 2.80, Glu: 2.20, Thr: 0.92, Cys: 0.85, Leu: 1.15.

IL-2Rβ(30-42): Asp: 1.90, Thr: 1.05, Ser: 1.05, Cys: 0.9, Glu: 1.15, Val: 0.95, His: 0.85, Ala: 1.05, Trp: 0.82, Pro: 1.10, Arg: 1.85.

IL-2Rβ(30-41): Asp: 1.95, Thr: 0.90, Ser: 1.15, Cys: 0.85, Glu: 0.95, Val: 1.10, His: 0.86, Ala: 1.10, Trp: 0.95, Pro: 0.95, Arg: 1.14.

Cell Cultures

Human peripheral blood lymphocytes (PBL) were obtained from normal donors and were isolated by density gradient centrifugation on Hypaque Ficoll (Flow Laboratories). Cells were washed twice with RPMI-1640 medium and resuspended herein with 10% (v/v) fetal calf serum (FCS), penicillin–streptomycin and L-glutamine. Lymphocytes were activated with phytohemagglutinin-P, 2 μg/ml, (PHA-P; Flow Laboratories) and incubated at 37 °C for about 72 h in a 5% CO_2 humidified atmosphere to generate PHA-blasts. The human T-cell line JURKAT-J6 was maintained in RPMI 1640 medium containing 10% FCS.

Radiolabelled IL-2 Binding Assay

Prior to the binding assay cells were incubated in RPMI-1640 at 37 °C for 45 min, in order to remove endogenously secreted and bound IL-2. Cells were then washed twice, and aliquots of $1.0 - 1.2 \cdot 10^6$ cells were resuspended in 50 μl of PBS containing 5% FCS and 1% BSA and incubated with 16 different concentrations (20 – 1 000 pmol/l) of human recombinant IL-2 radiolabelled with [^{125}I] (Amersham; specific activity > 600 μCi/mmol) at 4 °C for 1 h. When the binding assay was carried out in the presence of a synthetic peptide, cells or [^{125}I]IL-2 were preincubated with 500 nmol/l of the peptide at 4 °C for 1 h. Free and cell-bound radioactivity were separated by centrifugation (10 000 r.p.m., 2.5 min) over a hydrophobic layer consisting of 80% dibutyl phthalate (Merck) and 20% diisononyl phthalate (Sigma), and were measured in a γ-counter. Nonspecific binding was estimated by inclusion of a 1 000-fold excess of unlabelled human recombinant IL-2 (Cetus, U.S.A.). All sets of binding experiments were performed in duplicates. Binding assays in the presence and absence of each peptide were carried out in parallel in a total of three or four independent experiments. The number of binding sites per cell and dissociation constants (K_d) were evaluated by the method of Scatchard.

RESULTS AND DISCUSSION

Based on the primary structure and algorithms, such as hydrophilicity plot and secondary structure prediction, we have selected and synthesized peptides from the extracellular part of IL-2Rβ, that are likely to be involved in IL-2 binding. The hydrophilicity analysis performed, with the aid of Hopp and Woods computer program[22], has demonstrated that the IL-2Rβ sequence 35-45 corresponds to the highest average hydrophilicity value (Fig. 1). For the secondary structure we used a prediction scheme that combines the results of seven methods[23]. According to this approach a residue is considered likely to take α-helical, β-strand or turn conformation, if at least three methods predict it as either α-helical or β-strand or turn, respectively. As shown in Fig.

2, the region 30-50 is predicted to be a turn by five methods and above. Moreover, it contains several charged and highly polar amino acids that might give specificity to this site. Thus, eight peptides comprising residues 30-54, 35-54, 40-54, 44-54, 30-41, 30-42, 35-42 and 40-50 were synthesized (Table I).

To explore the function of the different synthetic peptides, first we studied their effect on [^{125}I]IL-2 binding on PHA-blasts. As shown in Fig. 3, the presence of the peptides IL-2Rβ(35-54), (30-42) and (30-54) produced a dose dependent increase of [^{125}I]IL-2 binding to PHA-blasts. This increase of [^{125}I]IL-2 binding was ranged between 100% and 175% at a saturating peptide concentration of 500 nmol/l after prein-

TABLE I
Amino acid sequence and biological activity enhancement of IL-2 binding by the synthetic IL-2Rβ peptides

Peptides	Sequence	Enhancement
30-54	D T S C Q V H A W P D R R R W N Q T C E L L P V S	+
35-54	V H A W P D R R R W N Q T C E L L P V S	+
40-54	D R R R W N Q T C E L L P V S	–
44-54	W N Q T C E L L P V S	–
40-50	V H A W P D R R R W N Q T C E L	–
30-42	D T S C Q V H A W P D R R	+
30-41	D T S C Q V H A W P D R	–
35-42	V H A W P D R R	–

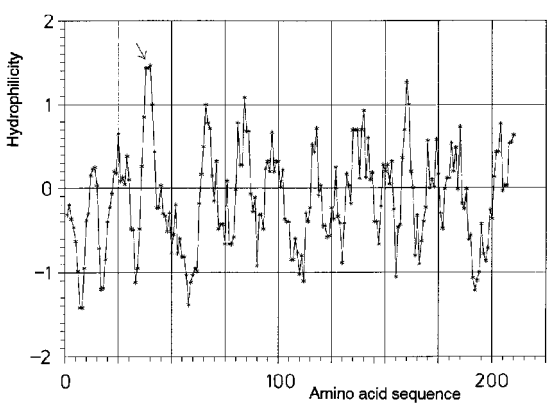

FIG. 1
Hydrophilicity plot analysis of the extracellular region of the IL-2Rβ chain. The arrow shows the maximum hydrophilicity value which corresponds to amino acids 35-45

cubation with constant concentration of [^{125}I]IL-2 (200 pmol/l). Conversely, the presence of the other peptides (IL-2Rβ(40-54), (44-54), (30-41), (35-42) and (40-50)) had no effect on IL-2 binding.

In an attempt to localize the minimum amino acid sequence responsible for the above effect we performed equilibrium binding experiments using two of the three active peptides, IL-2Rβ(35-54) and (30-42). Various concentrations of [^{125}I]IL-2 were pre-incubated with either IL2Rβ(35-54) or (30-42) before adding PHA-blasts. Scatchard plot analysis showed a 2- and 3-fold increase of the number of high and intermediate affinity IL-2 receptors, respectively (Table II and Table III). To exclude the possibility that peptide induces an enhanced nonspecific binding of IL-2 on the cells, we repeated similar experiments using a human T-cell line, JURKAT-J6, which does not constitu-

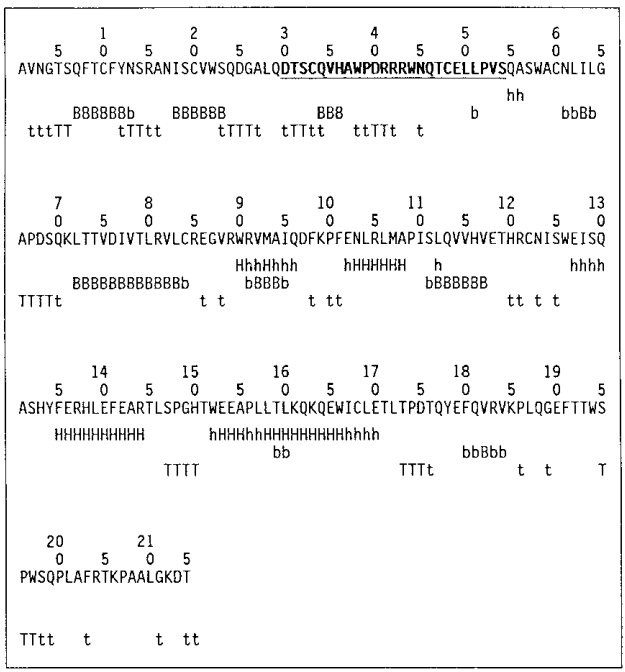

FIG. 2

Secondary structure prediction of the extracellular region of the IL-2Rβ molecule according to the JOINT program[23]. H, α-helix, B, β-strand and T, turn. Small letters indicate predictions by less than five methods and large letters indicate predictions by five methods and above. The selected region is underlined

tively express high-affinity IL-2R. No binding sites were observed both in the absence and presence of IL-2Rβ(35-54) and (30-42).

In order to examine whether the activity of IL-2Rβ peptides is mediated through their interaction with IL-2 or the membrane IL-2Rβ molecule IL-2Rβ(35-54) was preincubated with PHA-blasts and then [^{125}I]IL-2 was added. Scatchard analysis showed an insignificant increase in the number of high affinity IL-2R, while the number of intermediate affinity IL-2R was not modified (Table II, condition *B*). Same results were

TABLE II
Effect of the IL-2Rβ(35-54) on the IL-2 binding to PHA-blasts

Conditions	IL-2Rβ (35-54)	n [a]	High affinity[b]		Intermediate affinity[b]	
			K_d, pmol/l	Sites/cell	K_d, pmol/l	Sites/cell
A [c]	–	3	52 ± 4	1 138 ± 40	723 ± 158	3 416 ± 400
	+		89 ± 7	2 010 ± 130	968 ± 73	9 777 ± 552
B [d]	–	3	115 ± 11	1 463 ± 84	592 ± 136	2 740 ± 338
	+		137 ± 14	1 570 ± 125	615 ± 40	2 735 ± 160

[a] *n*, Number of independent experiments. [b] Results are mean ± SEM. [c] *A*, the peptide was preincubated with various concentrations of [^{125}I]IL-2 and then cells were added. According to the *t*-test[24] for a confidence interval of >95% ($P < 0.05$) the values of K_d and the number of receptors/cell increased in the presence of IL-2Rβ(35-54). [d] *B*, the peptide was preincubated with cells and then [^{125}I]IL-2 was added.

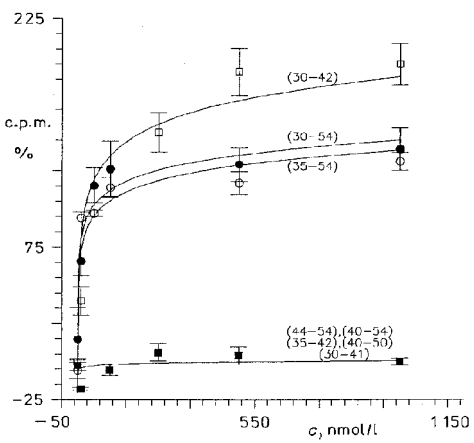

FIG. 3
Enhancement of [^{125}I]IL-2 binding (in c.p.m., %) to PHA-blasts in the presence of the synthetic peptides (*c*). Every point represents the mean value of duplicate assays. Error bars have been drawn from three independent determinations

obtained with IL-2Rβ(30-42) peptide under the above experimental conditions. Conversely, preincubation of the peptides with IL-2 increases the number of the binding sites per cell, Table II (condition *A*), and Table III. However, these sites can bind IL-2 with a lower affinity since, in all binding experiments performed the dissociation constant of high affinity was higher than that of the high affinity IL-2R detected in the absence of peptides. Moreover, the increase in the dissociation constant in the case of intermediate affinity receptors was such that these sites correspond to the low affinity receptors ($K_d \sim 1$ nmol/l).

Extensive studies on the IL-2 molecule active sites showed[3,25] that it contains several domains that are involved in binding to its receptor chains. Most of these domains are distinct for each chain[5,26]. Based on these results a likely interpretation of our findings is that synthetic peptides IL-2Rβ(35-54) and (30-42) probably interact with one of the domains on the IL-2 molecule which participates in IL-2Rβ binding. This interaction could induce conformational changes in IL-2 resulting to its binding on membrane chains which are not revealed under the usual experimental conditions.

Whatever the case the IL-2Rβ sequence VHAWPRR (35-42), which is the common domain among the three active peptides, seems to play a role in the interaction of IL-2 with its receptors. However, the peptide which is consisted of these seven amino acids did not demonstrate any biological effect (Fig. 3) suggesting that residues of the N- or C-terminus are also necessary to stabilize the active conformation of this region. It is interesting to note that a set of experiments using the peptides IL-2Rβ(35-54) and (40-54) with protected sulfydryl groups at Cys[48] gave the same results (data not shown). This finding indicates that the cysteine 48 might not be involved in IL-2 binding, at least under the experimental conditions described.

Although the mechanism of action of the synthetic peptides is unknown, the experimental system described raises several important questions concerning the partici-

TABLE III
Effect of IL-2Rβ(30-42) on the IL-2 binding to PHA-blasts

IL-2Rβ (30-42)	n [a]	High affinity[b]		Intermediate affinity[b]	
		K_d, pmol/l	Sites/cell	K_d, pmol/l	Sites/cell
–	4	68 ± 11	1 234 ± 116	374 ± 64	3 157 ± 285
+		150 ± 23	2 430 ± 278	1 130 ± 174	9 808 ± 1 135

[a] n, Number of independent experiments. [b] Results are mean ± SEM. The peptide was preincubated with various concentrations of [^{125}I]IL-2 and then cells were added. According to the *t*-test[24] for a confidence interval of >95% ($P < 0.05$) the values of K_d and the number of receptors/cell were increased in the presence of IL-2Rβ(30-42).

pation of different IL-2R chains and binding sites in the formation of IL-2/IL-2R complex. Synthetic peptides could be valuable tools in order to understand ligand interaction with its receptor as well as the formation of the high affinity IL-2R. Further experiments using various cell lines which exclusively express the α- or β-chain are presently in progress in our laboratory.

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