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Research paper

Characterization of linear mimetic peptides of Interleukin-22 from dissection of protein interfaces

Sara La Manna [a, 1], Pasqualina Liana Scognamiglio [a, 1], Concetta Di Natale [a], Marilisa Leone [b], Flavia Anna Mercurio [b], Anna Maria Malfitano [a], Francesca Cianfarani [c], Stefania Madonna [d], Sergio Caravella [d], Cristina Albanesi [d], Ettore Novellino [a], Dr. Daniela Marasco [a, *]

[a] Department of Pharmacy, CIRPEB: Centro Interuniversitario di Ricerca sui Peptidi Bioattivi, University of Naples "Federico II", 80134, Naples, Italy
[b] Institute of Biostructures and Bioimaging, CNR, 80134, Naples, Italy
[c] Laboratory of Cellular and Molecular Biology, Fondazione "Luigi Maria Monti", Istituto Dermopatico dell'Immacolata (IDI), IRCCS, Via Monti di Creta, 104, 00167, Rome, Italy
[d] Laboratory of Experimental Immunology, Fondazione "Luigi Maria Monti", Istituto Dermopatico dell'Immacolata (IDI), IRCCS, Via Monti di Creta, 104, 00167, Rome, Italy

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ABSTRACT

Interleukin-22 (IL-22) belongs to the family of IL-10 cytokines and is involved in a wide number of human diseases, including inflammatory disorders and cancer pathology. The ligand-receptor complex IL-22/IL-22R plays a key role in several pathways especially in the regulation and resolution of immune responses. The identification of novel compounds able to modulate IL-22/IL-22R complex could open the route to new therapeutic strategies in multiple human diseases.

In this study, we designed and characterized IL-22 derived peptides at protein interface regions: several sequences revealed able to interfere with the protein complex with IC_{50} in the micromolar range as evaluated through Surface Plasmon Resonance (SPR) experiments. Their conformational characterization was carried out through Circular Dichroism (CD) and Nuclear Magnetic Resonance (NMR) spectroscopies, shedding new light into the features of IL-22 fragments and on structural determinants of IL-22/IL-22R1 recognition. Finally, several peptides were tested on human keratinocyte cultures for evaluating their ability to mimic the activation of molecular pathways downstream to IL-22R in response to IL-22 binding.

1. Introduction

Many diseases, such as cancers chronic inflammation, wound healing and infections are characterized by the deregulation of cytokines' activities [1–3], thus the modulation of their signalling through synthetic compounds represents a valuable therapeutic approach in inflammatory diseases [4]. Interleukin (IL-22), belongs to the IL-10–interferon family [5,6] and, like all members, acts via a transmembrane receptor complex, named IL-22 receptor (IL-22R) [7]. Usually the recognition by IL-10-like proteins toward their receptors activates signal transduction pathways involved in gene expression or repression [8]. In contrast with other cytokines, IL-22 does not directly regulate the function of immune cells [9] but rather those at barriers, such as the skin and tissues of the digestive and respiratory organs, pancreas, liver, kidney: here it induces the expression of antibacterial proteins and chemokines. IL-22-binding protein (IL-22BP), is a receptor for IL-22 with single-chain, that strongly regulates IL-22 cellular effects [10,11]. X-ray diffraction data indicate that IL-22 is endowed with a bundle structure, constituted by six α-helices (A to F) with connecting loops (including preHA region), and three potential N-linked glycosylation sites, stabilized by two intramolecular disulphide bridges [12,13]. Biologically IL-22 acts as a monomer [14] even if no-

* Corresponding author. Department of Pharmacy, University "Federico II", Via Mezzocannone, 16, 80134, Naples, Italy.
E-mail address: daniela.marasco@unina.it (D. Marasco).
[1] These are co-first authors.

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covalent dimers and tetramers have been observed *in vitro*, at high concentrations [14]. IL-22R is a heterodimeric complex composed of IL-22R1 and IL-10R2 subunits, both containing intracellular, transmembrane and extracellular moieties [15]. STAT (signal transducer and activator of transcription) recruitment sites are represented by Tyr-X-X-Gln motifs that are contained four times in the intracellular portion of IL-10R2 that is shorter with respect to that of IL-22R1. The extracellular domains of IL-22R1 and IL-10R2 form two tandem domains: the N-terminal D1 and the D2 regions located in proximity of cell membrane [16]. IL-22/IL-22R interaction was proposed to be endowed with a multiphase mechanism: cytokine initially binds to IL-22R1 subunit, forming the IL-22/IL-22R1 complex. This complex induces a conformational change in IL22 protein and allows a secondary binding to IL-10R2 chain. Such formed ternary complex activates the JAK/STAT signalling pathway leading to diverse biological effects of IL-22 [17,18]: the phosphorylated IL-22R1 tyrosine residues interact with STATs both through a SH2-domain or its C-terminus coiled-coil regions [19]. Indeed, the phosphorylation of STAT3 at the Tyr705 is the main event observed in IL-22-treated cells, although weak activation of STAT1 and/or STAT5 could be observed [20,21]. These events enable STATs to dimerize and translocate into the nucleus: here they modulate their target genes' expression. In parallel to STATs, IL-22 can also trigger molecular pathways, including those leading to mitogen-activated protein kinase (MAPK)/extracellular signal regulated kinases (ERK)1/2 [22].

In the skin, human keratinocytes express IL-22R and, in pathological conditions, respond to IL-22, which induces the expression of several genes, especially those involved in the innate defence mechanisms, inhibits cellular differentiation and increases proliferation [23–25]. IL-22 induces the expression of certain chemokines and cellular mobility, and of several extracellular and intracellular proteins able to amplify in turn its effects [9]. In chronic inflammatory skin diseases, such as psoriasis, or in skin cancers, IL-22 pathway is aberrantly activated and many studies reported on the beneficial effects of the inhibition of IL-22 and/or IL-22R1 functions [26,27]. On the other hand, strengthening IL-22R1-mediated signalling might alleviate several other inflammatory conditions in ulcerative colitis, asthma pancreatitis and acute liver damage [28,29] [30]. Recently IL-22 was identified as a pro-healing factor of diabetic wounds, as it enhances re-epithelialization by promoting keratinocyte proliferation/de-differentiation, and by inducing VEGF production [31].

In this study, we designed and characterized IL-22 based peptides that were analyzed by competitive and direct binding assays through Surface Plasmon Resonance (SPR) experiments. Their conformational characterization by Circular Dichroism (CD) and Nuclear Magnetic Resonance (NMR) spectroscopies had shed new light into the intrinsic features of IL-22 fragments and structural determinants of IL-22/ILR1 recognition. Finally, selected IL-22 mimetic peptides were analyzed for their capability to activate molecular signalling dependent on IL-22R in human keratinocytes.

2. Materials and methods

2.1. Peptide synthesis

Solid phase peptide syntheses and purification were performed as already reported [32]. Briefly IL-22 derived peptides reported in Table 1 were synthesized employing the solid phase method on a 50 μmol scale following standard Fmoc strategies of SPPS [33].

RP-HPLC was employed for their purification applying a linear gradient of 0.1% TFA CH_3CN in 0.1% TFA water from 5% to 65% over 12 min using a semi-preparative 2.2 × 5 cm C18 column at a flow rate of 20 mL/min. LC-MS analysis confirmed their identity and purity.

2.2. Circular Dichroism (CD) spectroscopy

CD spectroscopy experiments were carried out by employing a Jasco J-810 spectropolarimeter (JASCO Corp, Milan, Italy) at room temperature in the interval scan 190–260 nm of wavelength. Shown spectra resulted from the average of three scans, to which spectra of correlated blanks were subtracted. CD signals were converted to mean residue ellipticity with $deg^{*}\ cm^{2*}dmol^{-1*}res^{-1}$ as units. Scan speed value was 20 nm/min, band width 2.0 nm, resolution at 0.2 nm, sensitivity at 50 mdeg, and response was set at 4 s. Peptides concentrations was always 100 μM in a quartz cuvette with a path-length of 0.1 cm (HF also at 1.2 mM in 0.01 cm path-length cuvette). Solvents were: 10 mM phosphate buffer (pH = 7.0) in volume mixtures with TFE (2,2,2 trifluoroethanol) and HFIP (1,1,1,3,3,3-Hexafluoro-2-propanol).

2.3. NMR analysis

NMR analysis was performed for preHA + HA-derived and HF peptides. Spectra were recorded in the temperature range 25–30 °C at 600 MHz on a Varian Unity Inova instrument with a cold probe. For the preHA + HA-derived peptide, three samples, with a total volume equal to 600 μL, were prepared a) 1.3 mM concentration in 10 mM sodium phosphate buffer pH = 7.2 with 5% (v/v) D_2O (99.8% d, Armar Scientific, Switzerland); b) 0.8 mM concentration in a mixture 10 mM sodium phosphate buffer pH = 7.2/2,2,2-trifluoroethanol-d3 (TFE >99.5% isotopic purity; Sigma-Aldrich, Milan-Italy) 60/40 (v/v); c) 0.8 mM concentration in a mixture 10 mM sodium phosphate buffer/TFE 40/60 (v/v).

NMR samples of HF sequence consisted of i) 600 μL of a mixture H_2O/1,1,1,3,3,3-Hexafluoro-2-propanol-d2 (HFIP, 99% D, Sigma-Aldrich, Milan, Italy) 50/50 v/v at a) 1.2 mM, and b) 0.4 mM, and of ii) 600 μL of 10 mM sodium phosphate buffer pH = 7.2 with 5% (v/v) D_2O at 1.1 mM.

The process of proton resonance assignment was conducted by analyzing 2D [^{1}H, ^{1}H] TOCSY (Total Correlation Spectroscopy) [34] (70 ms mixing time) and NOESY [35] (Nuclear Overhauser Enhancement Spectroscopy) spectra. The internal standard for

Table 1
Primary structure of IL-22 -based peptides designed and analyzed in this study.

IL-22 fragment	Peptide sequence	Name	MW (amu)	pI
41–73	RLDKSNFQQPYITNRTFMLAKEASLADNNTDVR	preHA + HA + AB_{LOOP}	3899	10.13
41–66	RLDKSNFQQPYITNRTFMLAKEASLA	preHA + HA	3084	10.75
41–53	RLDKSNFQQPYIT	preHA + HA derived	1650	10.37
49–66	QPYITNRTFMLAKEASLA	HA	2095	10.37
49–73	QPYITNRTFMLAKEASLADNNTDVR	HA + AB_{LOOP}	2910	7.19
67–73	DNNTDVR	AB_{LOOP}	874	3.71
157–179	ESGEIKAIGELDLLFMSLRNACI	HF	2564	4.04
114–129	YMQEVVPFLARLSNRL	HD	1977	10.93